Determining Phenotypic Traits Associated with Variability in Dietary Preferences in Grazing Sheep

A Thesis
Presented in Partial Fulfillment of the Requirements for the
Degree of Master of Science
with a
Major in Animal Science
in the
College of Graduate Studies
University of Idaho
by

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May 2020

Authorization to Submit Thesis

This thesis of Dillan Henslee, submitted for the degree of Master of Science with a Major in Animal Science and titled "Determining Phenotypic Traits Associated with Variability in Dietary Preferences in Grazing Sheep," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Sheep have the potential to be used globally as a grazing tool on rangelands for wildlife habitat improvement. Dietary preferences within sheep, especially preferences for consuming sagebrush, vary greatly. This thesis explores driving factors that could help explain variation in dietary preferences in sheep. We first examined bitterness avoidance in sheep by administering a gradient of concentrations of phenylthiocarbamide (PTC) dissolved in drinking water and quantifying individual intake in rams. We observed that sheep could detect PTC and that there was considerable variation in the concentrations at which PTC was avoided among sheep. These observations were similar to PTC avoidance described in humans, which has been attributed to genetic variations within type two taste receptors (Tas2r). Sheep have Tas2r genes, but due to the incomplete annotation of sheep Tas2r gene repertoire, extensive research studies correlating sheep Tas2r genes with phenotypic traits cannot be conducted. Using comparative genomic strategies, we proposed annotations for each of the non-annotated Tas2r genes in sheep, cattle and goat in order to complete the annotation of grazing livestock Tas2r repertoires. With the completed Tas2r repertoire of sheep, we will be able to continue our research with an extensive genetic study that may later be associated with dietary preferences in sheep. Taken altogether, the data from this research suggests that sheep can detect bitterness, which is likely a function of Tas2r genetic makeup and may be linked to sagebrush consumption. Better understanding of factors that contribute to dietary preferences in sheep could lead to selection for individuals that are uniquely suited for targeted grazing strategies that allow for sustainable grazing and dynamic wildlife habitat in sagebrush-steppe ecosystems.

Acknowledgements

Several individuals have helped me throughout my graduate program and words cannot express my appreciation and gratitude I have for them. My major professor, Dr. Ellison has helped me grow not only academically, but as a person. Her mentorship and guidance have proved invaluable and as an individual I will look up to for the remainder of my life. I thank Dr. B. Murdoch for her patience (with my many mistakes in her lab), time, love and support throughout my degree and for taking me in as one of her own. I thank Dr. Taylor for a warm welcoming at the U.S. Sheep Experiment Station. His knowledge and guidance will be carried and referred to throughout my career. I thank Dr. Yelich for his help in conducting experiments, where his experience was invaluable. Dr Yelich never thought twice about helping and was always there to assist me with the "grunt" work. I thank Dr. Hall for his time and knowledge on my committee. Lastly, I would like to thank Kim Davenport and Gabbi Becker for their contagious happiness and assistance.

Dedication

I would like to thank my friends and family for all their support, most importantly, my wife. The time and sacrifices my wife, Haley Henslee, has made in order to support my education is truly inspiring. The amount of love and encouragement I have received during my degree is will always be cherished and never forgotten. Lastly, I would like to thank my friends who assisted me inside and outside of graduate school: Kenny, Taylor, Ben, Marcus, Landon, Cale, Shawn, and Erick.

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Statement of Contribution

Avoidance of Phenylthiocarbamide in mature Targhee and Rambouillet rams

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Dillan Henslee's responsibilities included: Collecting and organizing data, conducting statistical analyses, constructing tables and figures, and writing, formatting, revising, and submitting the manuscript.

Joel Yelich's responsibilities included: Experimental design, collecting data, assisting with statistical analyses, and revising the manuscript.

J. Bret Taylor's responsibilities included: Experimental design, financial support, formulating PTC solution, and revising the manuscript.

Melinda Ellison's responsibilities included: Experimental design, financial support, revising the manuscript.

Chapter 1: Literature Review

Seas of Sagebrush

Much of the western U.S. is covered in vast seas of sagebrush (Whitson and Alley, 1984). Sagebrush is a key component of the range ecosystem because it provides multi-use characteristics for wildlife and helps to stabilize soils (Vale, 1974). Several wildlife species, such as deer, sage-grouse, antelope, elk, and rabbits not only use sagebrush as a forage, but also for protection (Martin, 1970; Wright and Bailey, 1982). Sagebrush is a very diverse woody shrub species that can tolerate very harsh climates (USDA, 2019). Sagebrush plants grow 0.6 – 4 m tall (depending on species and topography) and are typically found in high desert regions that receive little rainfall (20 – 76 cm) from 600 – 2,100 m in elevation, where ambient temperatures exceed upwards of 37.7° C in the summer and plummet below -17.7° C in the winter (USDA, 2019). Given the wide range of attributes in which it can thrive, sagebrush is a very resilient shrub, and without invasion of noxious weeds or annual grasses, can easily establish or regenerate itself following disturbance (Davies et al., 2011; USDA, 2019).

There are several species of sagebrush, and because hybridization often occurs, species identification may be challenging (McArthur et al., 1988; Wang et al., 1997). Individual species tend to favor specific elevations, climates, and annual rainfall, but ideal conditions have a high degree of overlap among species (Mahalovich and McArthur, 2004; USDA, 2019). Sagebrush tends to decrease in size as elevation increases, which is associated with a moisture gradient, where moisture availability decreases as elevation increases (Mahalovich and McArthur, 2004).

Sagebrush have many recognizable attributes, but the smell is arguably the most distinguishable attribute. It contains a very pungent fragrance, most evident after rain, which is due to the essential oils found in sagebrush (Adams and Oakberg, 1934; Kinney et al., 1941). In particular, camphor is a predominant terpenoid in sagebrush that is very fragrant (Adams and Oakberg, 1934; Kinney et al., 1941). Terpenoids are phytotoxins produced by plants that elicit strong odors and a

bitter taste upon mastication to deter grazers from consuming the plant (Johnson et al., 1985; Glendinning, 1994). Although sagebrush contains terpenoids that grazers may avoid, sagebrush is a vital forage, especially during winter months, for several wildlife species and is highly preferred during that timeframe (White et al., 1982; Welch and Wagstaff, 1992; Connelly et al., 2000). While, sagebrush is often termed "a forage of last choice" for grazing livestock due to low palatability (Nagy et al., 1964; Nagy and Tengerdy, 1968), it is relatively high in crude protein (11.7 – 12.6% CP) compared with other rangeland plants. Sagebrush has an *in-vitro* dry matter digestibility (IVDMD) of 57.8 – 58.1%, which would make it an excellent winter feed source for grazing livestock (Welch and Wagstaff, 1992; Welch, 2005).

Shrub Encroachment

Shrub encroachment is a worldwide issue. In the western United States, one of those shrubs is mountain big sagebrush (*Artemisia Tridentata*) (Wambolt and Payne, 1986; Johnson et al., 1996). As sagebrush becomes overgrown within an ecosystem, it has been associated with decreased plant diversity, forage biomass production, and sagebrush understory production, which in turn decreases wildlife habitat (Launchbaugh, 2003). Nature has accounted for this ecological shift with a natural fire interval cycle that rejuvenates rangelands (Miller and Rose, 1999). In more recent years at lower elevations, invasion of exotic annual grasses has become common post-fire, leading to shifts in plant communities and decreased sagebrush regeneration (Knapp, 1996). Restoration of plant communities after invasion of exotic annual grasses is expensive and often fails (D Antonio et al., 2001). In fear of exotic grass invasion, fire is often considered a negative action on rangelands. Due to human interactions, some of these rangelands have not burned for several years, or decades, past their respective fire interval life cycle (Miller and Rose, 1999).

Sagebrush is an essential component of western U.S. rangeland ecosystems. Sagebrush can be very beneficial to wildlife by providing cover and forage (Castrale, 1982; Wright and Bailey, 1982), but it can also be a detriment to wildlife habitat if it chokes out

important plant species resulting in reduced plant diversity (Frischknecht and Baker, 1972; Launchbaugh, 2003). In sagebrush-steppe rangelands, several methods of sagebrush removal and suppression have been implemented, but all come with limitations and drawbacks (Wambolt and Payne, 1986). Although very resilient, sagebrush cannot tolerate being over-watered (USDA, 2019). Ranchers and farmers within sagebrush-steppe regions have reported that the easiest method for removal of sagebrush is by watering the shrub until it perishes (USDA, 2019; Communication). However, this method is only feasible if an irrigation system is present. Additionally, mechanical methods are expensive, area-limiting, and disturb the whole ecosystem (Wambolt and Payne, 1986). Tebuthiuron is a herbicide that has been used successfully to control sagebrush (Johnson et al., 1996), but the high product and application costs makes it an invalid solution for treatment of vast rangelands areas. Finally, grazing of livestock is known to affect sagebrush ecosystems, but varies widely based upon management (Davies et al., 2011).

Sheep Sagebrush Consumption

Near-infrared spectroscopy uses different wavelength of light that is not visible with the naked eye to determine the composition of objects. Using near-infrared spectroscopy (NIRS) for fecal composition analyses, sagebrush consumption in sheep was variable (10 - 40%) (Snowder et al., 2001). Factors that contribute to the considerable variation in sagebrush consumption by sheep are not well understood. One theory proposes that sagebrush consumption by some livestock is a learned behavior. Launchbaugh et al. (2001) suggested that if juvenile sheep (under the age of one year) have never been exposed to consuming sagebrush, sagebrush will likely not be part of their feeding ecology in adulthood. Juvenile exposure to consuming sagebrush may come from watching and/or mimicking their dam or other flock members. Similarly, for cattle that graze on rangeland, Zimmerman (1980) proposed that it is important for suckling calves to learn from their mothers' which shrubs to graze. Nolte and Provenza (1992) described learned behaviors in sheep, including

how experiences as a juvenile affected dietary preferences later in adulthood. Orphaned lambs (2-3 days of age) were segregated into two groups and fed either garlic-flavored milk or onion-flavored milk for 50 days. Lambs later preferred rations made up of feeds with the same flavor of milk they had received. This observation suggested that flavors associated with the milk consumed by lambs while suckling may influence forage preferences as an adult (Nolte and Provenza, 1992).

Learned behaviors may also be driven by individual experiences of the animals. It has been reported that sheep experienced with grazing on rangelands consumed considerably more sagebrush than in experienced sheep (Narjisse, 1981). Sheep are known to be adaptive grazers and studies have suggested that sheep can also be trained to consume forages (Provenza and Balph, 1987; Launchbaugh, 2003).

Although learned behaviors in sheep are likely part of what drives an individual's dietary preferences in consumption of sagebrush, it is probably not the only factor. Another theory proposes that the variability in sagebrush consumption observed in sheep is due to the bitterness found in sagebrush (Yabann et al., 1987). The bitterness of sagebrush can be attributed to the presence of monoterpenoids found in sagebrush, which are elicited upon mastication of the sagebrush and serve as a plant defense mechanism to deter grazers, along with its' pungent odor (Cedarleaf et al., 1983). In plants, bitterness is often associated with toxicity (Garcia and Hankins, 1975). Ungulates have the ability to detect a wide variety of bitter-tasting compounds and use this ability to avoid ingestion of toxic plants (Glendinning, 1994). Although the ability of ungulates to detect bitterness is not well-understood, variations within an individual's ability to detect bitterness could play a role in the variability in sagebrush consumption observed in sheep.

Research performed by Vale in 1974 suggests that animals that rely on sagebrush for forage have adapted mechanisms that allow them to suppress the toxins that sagebrush produces. Launchbaugh et al. (2001) proposed that grazing animals that utilize sagebrush may be able to identify the presence of phytotoxins through taste, smell and/or gustatory responses. Studies have

been conducted to determine the specific chemical compositions for sub-species of sagebrush, which have yielded variable results within small and large spatial scales (Welch et al., 1983). However, more information is needed to better understand the specific mechanisms that may contribute to sagebrush consumption, or lack thereof, by grazing ruminants.

Yabban et al. (1987) observed free-grazing sheep in rangelands consisting of primarily mountain big sagebrush and they suggested that it was monoterpenoid content that influenced the consumption of sagebrush, not dietary requirements. If dietary requirements were the sole factor driving sagebrush consumption, sheep would be indiscriminate towards the sagebrush they chose to browse. However, it was instead observed that sheep selected sagebrush with total monoterpenoid content much lower in comparison to that of the plants they rejected. Monoterpenoid content of plants rejected was 2.6 times greater than plants selected (Yabban et al., 1987).

Another interesting observation made by Yabban et al. (1987) was that sheep preferred older sagebrush plants compared to the younger, smaller plants which mainly consisted of green vegetation. Of the older plants, Yabban et al. (1987) observed that sheep consumed the previous year's growth and would bite the plant precisely so the new year's growth would fall on the ground. Although, the new year's growth was much greater in IVDMD and CP, it was also greater in monoterpenoid concentration (Yabann et al., 1987). This further supports the contention that the level of monoterpenoids drive sagebrush selection, instead of nutritional quality. Provenza and Malechek (1984) also observed a similar behavior in goats grazing black brush (*Coleogyne ramosissima*), where they consumed older growth and left the new year’s growth, which was greater in tannin content.

Variability in sagebrush monoterpenoid concentration was also noted by Narjisse (1981) when sheep and goats were given the option of feed with or without monoterpenoids. Sheep avoided feeds with monoterpenoids at the beginning of the trial and transitioned to indiscriminate consumption by the end of the trial, while goats were indiscriminate throughout the trial. A subsequent test was conducted where anosmic (smell blindness) sheep and goats were offered the

same feed options. The sheep were indiscriminate throughout the trial, while the goats avoided the feed that contained monoterpenoids (Narjisse, 1981). This observation suggests that sheep and goats may have been using different biological mechanisms to identify presence of toxins. Wright (1970) also observed variability in consumption of three-tip sagebrush (*Artemisia tripartita*) in which the plants with the lowest monoterpenoid content were grazed so heavily by sheep that the plants perished, and the plants with high monoterpenoid content were untouched. There are several conflicting studies that either suggest a correlation between consumption of sagebrush and monoterpenoid concentration (Wright, 1970; Narjisse, 1981 (sheep); Yabann et al., 1987) or suggest no correlation (Narjisse, 1981 (goat); White et al., 1982; Welch et al., 1983). Aside from taste and smell, several other mechanisms could play a role in monoterpenoid consumption, such as their ability to detoxify and the nutritional plain of diet (Dziba et al., 2007).

Differences in monoterpenoid production in sagebrush is loosely understood. There are several probable factors that could affect monoterpenoid production in sagebrush, including disturbance response, genetic makeup, and season (Karban et al., 2006). Disturbance can occur from grazing and/or breakage of a plant. It was proposed that sagebrush plants can communicate by eliciting volatile cues after being grazed, which triggers neighboring plants to increase their defense mechanisms, such as increasing phytotoxin production (Karban et al., 2006). Better plant survival rates have been associated with greater phytotoxin production (Wright, 1970). Time of season is also a known factor of changes in phytotoxin production. For example, phytotoxin production in sagebrush increases going into the fall, which could be driven by a decrease in soil moisture or a potential mechanism developed by sagebrush to increase phytotoxin levels in the season of greatest grazing pressure (fall/winter) (Cedarleaf et al., 1983).

Snowder et al., (2001) analyzed fecal samples from 549 Rambouillet ewes to determine sagebrush composition in their diet. Their findings suggested that sagebrush consumption in sheep was highly variable, ranging from 10% – 40% (Snowder et al., 2001). In efforts to understand if sheep

could be used to control encroachment of sagebrush, Seefeldt (2005) expanded upon the work by Snowder et al., (2001) using the same population of ewes, where the ewes were stratified into either a high preference or a low preference group. After grazing pastures of overgrown sagebrush (canopy > 33%), the high preference group consumed substantially more sagebrush than the low preference group, but no differences in canopy reduction were observed between the groups (Seefeldt, 2005). Because sagebrush is a slow growing, woody shrub in which the young soft growth that sheep can consume grows vertically instead of horizontally, consumption of the plant may not be observed by measuring canopy (a measurement of the diameter of the plant). Additionally, sheep only have one row of incisors, which makes reduction of the older, woody parts of the plant difficult due to the anatomy of their mouth.

Bitterness Avoidance in Sheep

There are five known taste senses that determine dietary preferences or avoidances based on how an individual biologically perceives each of the senses. Of the five taste senses: bitter, sweet, sour, salty, and umami; bitterness is the most sensitive taste sense in sheep (Goatcher and Church, 1970). Goatcher and Church (1970) administered increasing concentrations of quinine, a bitter tasting chemical, in water to groups of sheep (n = 5) and observed an inverse relationship between quinine concentration and total solution consumption. As the concentration of quinine increased, the total consumption of solution decreased. Although not measured on an individual basis, avoidance of increasing bitterness was an interesting observation, suggesting that some individuals may be willing to consume bitter-tasting plants if the bitterness intensity is low enough.

In humans, taste preference is highly variable between individuals (Drayna et al., 2003), and the variability has been linked to type two taste receptors (Tas2r), specifically the *TAS2R38* gene (Kim et al., 2003). Because the *TAS2R38* gene is also present in sheep (Ferreira et al., 2013), a similar variability in taste preference is likely to be observed in sheep. Taste preference is highly complex and has been suggested to be driven by several factors, including genetics, age, health, post-ingestive

feedback, and learned behaviors (Tepper, 2008). Taste is the first mechanism used to determine acceptance or avoidance of feed (Bachmanov and Beauchamp, 2007). Individual intake of certain feeds has been theorized to be adjusted based upon the taste and post-ingestive feedback (Provenza et al., 1992).

Launchbaugh et al. (1993) implemented a series of experiments where different concentrations of lithium chloride (LiCL) were added to oats and fed to orphan lambs, which were visually observed to determine the manner in which they regulated feed intake. Initially, lambs were split into three groups and fed oats with either a low, medium, or high dosage of LiCL. The high dosage group exhibited the lowest intake while the low dosage group had the greatest intake. Subsequently, all groups were fed the medium dosage of LiCL, and the lambs that initially received a low dose of LiCL exhibited decreased feed intake while lambs that initially received a high dose of LiCL had a greater feed intake, which suggested that lambs were able to regulate their intake based on taste and bitterness intensity (Launchbaugh et al., 1993).

In the same study, Launchbaugh et al. (1993) conducted another experiment where lambs were offered barley in the afternoon for 10 days and received an oral capsule with a dosage of LiCL based on the percentage of barley intake. Lambs were segregated into three groups for LiCL administration: high, medium, and variable. The high dosage group received a dose of LiCL administered as a rate of 2.25% of barley consumed, the medium group received a dose of LiCL administered as a rate of 1.5% of barley consumed, and the variable group received a random dose of LiCL 0.75, 1.5, or 2.25% of barley consumed. Over the course of the experiment, the medium group consumed the most barley, whereas barley intake for the high and variable groups did not differ. The variable group adjusted their intake accordingly to the dosage received, likely to prevent toxic overdose, even though the average dosage received was equivalent to that of the medium group (Launchbaugh et al., 1993). Collectively, the studies by Launchbaugh et al. (1993) suggested that sheep use several mechanisms to avoid toxic ingestion. One method of regulation of feed intake is not

only based on perceived bitterness intensity, but also according to post-ingestive feedback and the association of taste to that feed. Flavor is suggested to regulate intake based upon post-ingestive feedback (Launchbaugh et al., 1993). Similarly, sheep grazing on rangelands likely regulate their intake of a plant relative to the perceived bitterness intensity of that plant and/or the post-ingestive feedback associated with consuming that plant. Because bitterness is known to vary in sagebrush, sheep may regulate intake of sagebrush according to perceived bitterness intensity of each sagebrush plant they consume, or relative to a past experience associated with consuming sagebrush (Launchbaugh and Provenza, 1994).

In another similar study, Valliba et al. (2006) mixed forages with varying concentrations of monoterpenoids and administered them to groups (n = 8) of lambs. As the concentration of monoterpenoids increased, forage intake decreased. It was also noted that the time spent at the feeding rack increased as the monoterpenoid concentration increased (Villalba et al., 2006). Similarly, sheep have been observed to slow their feed intake in order to limit ingestion of toxic substances until post-ingestive feedback can be determined (Provenza et al., 1992). Slowing feed intake has also been observed when sheep are sampling novel feeds, possibly to prevent a toxic overdose that could cause death (Launchbaugh et al., 2001).

Balancing positive and negative digestive consequences (post-ingestive feedback) likely plays a role in determining acceptance and consumption of feeds (Launchbaugh et al., 2001). Generally, preference for a feed is developed when the taste is favorable and nutrient retention and uptake is adequate post-consumption. However, if the animal experiences illness or digestive upset, possibly caused by toxins in the consumed plant, it may develop an aversion to that plant. Therefore, the initial value of a novel plant or feedstuff may be driven by that individual's experiences associated with feeds of similar taste consumed in the past (Launchbaugh et al., 2001). Consumption has also been theorized to be driven by plant availability and the animal's nutritional status (ie., negative to positive energy balance), where an individual may seek out plants that meet nutritional

needs at that given time (Provenza, 1995). Perceived reward may transcend the negative effects of digestive consequences, especially those associated with toxic plants, which in some cases may result in animal health complications or death. Additionally, toxic effects that lead to death may occur before the animal can experience digestive consequences and adjust intake behavior accordingly. Therefore, the ability to detect toxins is essential for survival.

Some plants vary in toxicity so greatly throughout the year, that even experienced grazers are at risk of toxic ingestion. One plant that is prevalent in the Intermountain West of the United States and is likely the most well-known to sheep producers to be toxic and cause death is the *Lupinus* genus (hereafter, lupine; Cronquist et al., 1977). Identifying lupine species can be challenging because the plants crossbreed extensively and they contain no natural barriers against in-breeding (Cronquist et al., 1977). Lupine can become highly toxic in late-summer when the plant matures and produces highly toxic seeds that contain alkaloids (Keeler, 1976). However, the toxic seed trait is not carried by all lupine species (Cronquist et al., 1977). It is unknown whether sheep can detect alkaloids present in lupine; however, this plant material can be nutritionally valuable for sheep and is less toxic during certain times of the year (i.e. spring), which may give individuals a false sense of safety after grazing lupine early before it matures with no digestive consequences (Keeler, 1976). The most current recommended practices to avoid toxic death in sheep is to avoid lupine fields during the summers and/or complete toxicology assessment of the lupine to determine toxicity of lupine plants in the pasture (Panter et al., 2017).

Phenylthiocarbamide (PTC)

Studies have documented that grazing ungulates avoid bitter-tasting plants (Schwartz and Ellis, 1981; Yabann et al., 1987), but this trait for survival is not unique to ungulates (Kim et al., 2003). Nelson (2003) conducted an experiment, similar to the quinine study conducted by Church and Goatcher (1970), where PTC was administered in water to individually housed mice while monitoring the avoidance response. Similar to the response observed in sheep administered quinine (Church and

Goatcher, 1970), as PTC concentration increased, less solution was consumed (Nelson et al., 2003). Moreover, Nelson (2003) noted that avoidance of PTC did not occur at the same concentration for each individual mouse, which was similar to observations in human studies (Fox, 1932).

Phenylthiocarbamide avoidance in humans has been extensively researched since the accidental discovery of "tasters" and "non-tasters" in 1932 by Dr. Arthur Fox. Dr. Fox was a scientist for DuPont, where he accidentally spilled some PTC while working in the lab. His fellow scientists immediately started to complain of the bitter taste on their lips from the PTC crystals in the air. Dr. Fox did not taste anything bitter; in fact, he could not taste anything at all even though PTC had spilled most closely to him. This incident sparked his curiosity and led him to convince other scientists within the lab to test if they could taste the PTC, and subsequently his family and friends. Some individuals could not taste anything, like Dr. Fox (non-tasters), and others perceived it as extremely bitter (tasters) (Blakeslee, 1932; Fox, 1932).

Dr. Fox later published his findings; however, it was inconclusive which biological drivers were associated with the difference between tasters and non-tasters. He considered gender, race, and age, but found no connection. He theorized that because PTC was difficult to dissolve into a solution, the individuals that perceived PTC as bitter may have had a protein in their saliva that was able to solubilize PTC (Fox, 1932). Although more recent research made the discovery of the genetic control of being able to taste PTC (Kim et al., 2003), the original research led to an influx of interest from the scientific community that is still ongoing today.

Blakeslee (1932) studied PTC blindness in humans by examining the point of detection and the intensity perceived at that point. Later, Blakeslee and Salmon (1935) discussed the limitations of PTC testing, suggesting that using thresholds to determine "tasters" and "non-tasters" was an arbitrary method. Because a population's detection of PTC was linear, with no natural breaking point, two individual's detection of PTC may be at a similar concentration, however, they would segregate differently if one was just above the threshold and the other was just below the threshold. Another

limitation to PTC testing was that once PTC was detected, there was no scale with which to measure the intensity of bitterness perception. For some individuals, once PTC was detected it was perceived as low bitterness intensity, while some individuals could not actually taste bitterness, but they noted it was different than water. Furthermore, for some individuals, the perceived bitterness intensity increased as the concentration increased, while other individuals stated that once PTC was detected, they perceived it as extremely bitter regardless of the concentration (Blakeslee and Salmon, 1935).

Harris and Kalmus (1949) addressed the limitations outlined by Blakeslee and Salmon (1935) by administering PTC concentrations in a decreasing design, instead of administering PTC in increasing concentrations, where the test subjects received decreasing concentrations until they perceived the solution as water. Harris and Kalmus (1949) eventually compared the results of their study to other studies with similar demographic populations. The results followed a similar trend, but the thresholds at which PTC was no longer perceived were different, which was attributed to the lack of standardized methods for dissolving PTC into a solution (Harris and Kalmus, 1949). The Harris and Kalmus (1949) method of PTC testing was widely adopted within the scientific community as the correct method for testing PTC blindness. Lack of standardization has been attributed to being the largest single factor for inconclusive results of PTC thresholds determining “tasters” and “non-tasters” (Tepper, 2008).

It should be noted that “taste-test” using PTC was concerning because the chemical can be toxic in humans, so many of studies began substituting PTC with 6-n-propylthiouracil (PROP), a chemical that is very similar in structure to PTC. 6-n-propylthiouracil has been demonstrated to produce similar taste blindness reactions among individuals similar to PTC (Bartoshuk et al., 1994). Avoidance of PTC and PROP in humans has been reported as highly repeatable trait ($r = 0.75$ to 0.85; Harris and Kalmus, 1949, Keller and Tepper, 2004)

Additionally, Boyd (1950) discovered l-5-vinyl-2-thiooxazolidone, another chemical that elicited a similar taste reaction to that observed with PTC. A naturally occurring substance in foods

such as turnips, l-5-vinyl-2-thiooxazolidone is also known to cause goiter in humans (Shepard, 1961). Through this discovery, Boyd (1950) was one of the earliest scientists to suggest that genes may be adapted through evolution to prevent over-consumption of goitrogens. Others later demonstrated that PROP avoidance was not only correlated to goitrogen consumption, but food preferences in general (Fischer et al., 1961; Glanville and Kaplan, 1965). An interesting observation was made by Fischer et al. (1966), in that PTC tasters tended to have ectomorph body types and non-tasters tended to have endomorph body types. This observation sparked much attention throughout the scientific community to investigate how genes related to taste are not only associated with dietary preferences, but may also be expressed by other phenotypes.

Avoidance of PTC/PROP originally categorized humans as “tasters” and “non-tasters” until a sub-group of tasters called “super-tasters” were suggested to be able to detect PTC/PROP at very low concentrations and perceive them as intensely bitter (Bartoshuk et al., 1994). This subset of super-tasters also perceived more intense flavors in sweet foods and reported more irritation from the burning of alcohol. Interestingly, tasters and super tasters of PTC/PROP tended to perceive more bitterness from beer (Intranuovo and Powers, 1998) and more irritation from alcohol (Duffy et al., 2004). Tasting of PTC/PROP has even been negatively correlated with alcoholism (Pelchat and Danowski, 1992; DiCarlo and Powers, 1998). Another significant discovery was noted by Bartoshuk et al., (1994) was that the density of taste receptors on the tongue was significantly correlated ($r = 0.84$; $P < 0.001$) with the perceived intensity of PTC/PROP, which was later supported by Essick et al., (2003).

The frontier studies investigating taste blindness to PTC suggested it was a Mendelian simple recessive trait (Blakeslee, 1932; Fox, 1932). Phenylthiocarbamide testing was even used in parentage testing prior to the development of DNA parentage test technology (Tepper, 2008). However, as reports of “non-taster” parents giving rise to children that were “tasters” became more frequent, the Mendelian simple recessive trait theory was argued and dispelled (Das, 1958). Over time, it has

become accepted that taste blindness to PTC is not a Mendelian recessive trait, but rather a much more complicated trait controlled by a variation within a type two taste receptor.

Type Two Taste Receptors

Type two taste receptors (Tas2r) are 7-transmembrane G-protein coupled receptors and are the only taste receptors that perceive bitter taste (Adler et al., 2000). The orientation of the G-protein determines how well ligands can bind to it and the strength of the signal sent through the nervous system to the brain. Orientation of the G-protein can differ due to genetic variations, or single-nucleotide polymorphisms (SNP), within a Tas2r gene, which can drive variations in avoidance of certain chemicals observed among individuals (Kim et al., 2003). The Tas2r genes can be found not only within papillae of the tongue, but also throughout the gastrointestinal (GI) tract in enteroendocrine cells (Chandrashekar et al., 2000). This is significant since mastication and digestion break down chemical compounds into secondary metabolites, which can be highly toxic. The Tas2r genes found throughout the GI tract have been suggested to be involved in molecular pathways of protective responses, such as vomiting, which typically occurs after ingestion of toxins or poisons (Sternini et al., 2008). Although vomiting is not a mechanism that is not observed in sheep, it is likely substituted with slower sampling of novel feeds (Launchbaugh et al., 2001).

Type two taste receptors genes are orthologous and have only been extensively studied in humans and mice. Interestingly, Tas2r genes are relatively easy to identify in species' genomes because they predominantly cluster to specific regions, within comparable synteny blocks, and do not contain introns within the coding regions (Conte et al., 2002). The Tas2r gene repertoires are thought to be complete in humans and mice and are quite different between the two species. Human Tas2r genes tend to cluster to two specific regions on chromosome 7 and 12, while mice tend to cluster all Tas2r genes on chromosome 6 (Conte et al., 2002; Conte et al., 2003). Mice also have 10 more Tas2r genes than humans. It has been theorized that Tas2r gene repertoires and their function are shaped by the feeding ecology specific to each species (Dong et al., 2009). For example, it is not surprising mice

have such a large number of Tas2r genes because their diet largely depends on food availability. Rodents are known to forage on rummage and waste, which may be chemically diverse; therefore, survival in rodents is largely dependent upon ability to detect a wide range of toxins (Lush, 1986; Nelson et al., 2003). Human diets tend not to be as chemically diverse, and consequently, they have fewer Tas2r genes than mice.

Different Tas2r genes have the innate ability to detect certain ligands (Chandrashekar et al., 2000). Within a species, ligand detection may be the difference between life and death, therefore, each Tas2r gene that detects a certain ligand would be of great importance to the species and would be passed on to offspring (Go, 2006; Dong et al., 2009; Davis et al., 2010). Similarly, detection of a ligand not found within the feeding ecology of a species would be of non-importance and would likely be lost over time (Go et al., 2005). Gene loss and gain over time is commonly observed within vertebrates (Go et al., 2005; Go, 2006). Some Tas2r genes can be finely tuned to recognize one chemical that is of great importance, likely a chemical that is encountered often (Shi et al., 2003; Drayna, 2005). Chemicals that are encountered often can either be completely avoided (i.e., high toxicity) or ingested in limited quantities (i.e., low toxicity). Other Tas2r genes may be broad receptors, with the responsibility of detecting novel chemicals (Shi et al., 2003).

Of the Tas2r genes, variants within *TAS2R38* have been attributed to PTC avoidance (Kim et al., 2003). Hence, the *TAS2R38* gene is commonly referred to as the "PTC gene". Kim et al. (2003) discovered several possible haplotypes within the *TAS2R38* gene and in particular three SNP that would alter the amino acid sequences. These SNP occur at amino acid 49 resulting in a Proline to Alanine, 262 Alanine to Valine, and 296 Valine to Isoleucine substitutions. Of the possible haplotypes, two of them are observed in 96% of European descendant population and determine if an individual is a "taster" (PAV) or a "non-taster" (AVI) of PTC (Kim et al., 2003). Additionally more rare haplotypes, specific to sub-Saharan Africans, have also been discovered (Wooding et al., 2004). Further research is needed to understand if the less common haplotypes are classified in PTC

avoidance. Research related to PTC avoidance and Tas2r genes has been extensively studied in humans an effort to correlate avoidance with a number of traits. These studies have correlated PTC avoidance with dietary preference, substance abuse, body mass index, alcohol dependence, and nicotine use (Intranuovo and Powers, 1998; Duffy et al., 2004; Keller and Tepper, 2004; Wooding et al., 2004).

Grazing livestock (sheep, cattle, and goats) have fewer Tas2r genes than humans (25), typically 20 in sheep and 23 in cattle and goats. Although several Tas2r genes have not been annotated in sheep, cattle, and goats, the three species likely have high Tas2r gene similarity due to similar feeding ecology (Dong et al., 2009). The Tas2r clusters are found on chromosome 3 and 4 in sheep, chromosome 4 and 5 in cattle, and chromosome 4 and 5 in goat (NCBI, 2019). Although one of the clusters in sheep is found on a different chromosome than cattle and goat, it is interesting to note that this cluster in sheep is found on a metacentric chromosome and cattle and goats only have acrocentric chromosomes. The Tas2r clusters located within a similar region in sheep, cattle, and goat genomes gives credence to the theory that species of similar feeding ecology have similar Tas2r repertoires (Dong et al., 2009). Of the two Tas2r clusters, Cluster 1 in sheep, cattle, and goats is well annotated, with only one non-annotated gene in all three species. The nearly complete annotation of Cluster 1 for sheep, cattle, and goats is likely due to the high similarity to Cluster 1 found in humans. The Tas2r genes found within Cluster 1 are the same for sheep, cattle, goat, and humans with the exception that all of the human Tas2r genes in Cluster 1 have been annotated. Many of the Tas2r genes in Cluster 2 () have not been annotated in sheep (six of ten genes), cattle (two of thirteen genes), and goats (ten of thirteen genes), which makes comparing Cluster 2 across species difficult. The incomplete annotation of these genes restricts extensive research in how Tas2r genes can influence diet selection in grazing animals. (NCBI, 2019)

It stands to reason that humans and mice have more Tas2r genes than grazing livestock because humans and mice are omnivores and would likely come into contact with similar chemical

compounds in their feed with the addition of carnivorous-associated compounds which comparatively grazing livestock would not. There has been no research to-date that investigates correlations between Tas2r genes and phenotypic traits within ungulates. It is likely that Tas2r associations with phenotype are similar for grazing livestock to those observed in humans and mice, such as dietary preferences and body mass index (body condition in grazing livestock). However, the role of Tas2r genes in ungulates is a novel concept and little research has been conducted to-date. The majority of Tas2r genes in sheep, cattle, and goats have not been annotated, the completed annotation of these Tas2r repertoires would allow for extensive research to identify the role that each Tas2r gene may have on phenotype is constrained.

Chapter 2: Avoidance of Phenylthiocarbamide in Mature Targhee and Rambouillet Rams

Translational Animal Science. 2019. 4:1194-1204.

Abstract

Shrub encroachment on grasslands is a worldwide issue and sheep are a potential tool for mitigating shrub encroachment. Many shrubs, however, contain bitter-tasting compounds that may deter grazers. Cattle and sheep commonly graze rangelands, but of the two, sheep have a greater tolerance for bitter compounds and would be expected to consume more bitter-tasting vegetation. We hypothesized that sheep could detect (i.e., taste) bitter-tasting compounds and the sensitivity to these compounds would vary from animal to animal. The objective of this study was to determine whether sheep could detect the bitter-tasting compound phenylthiocarbamide (PTC), and if so, what PTC concentration would elicit an avoidance response. Using a crossover study design, mature Rambouillet and Targhee rams (n = 30) were subjected in randomized order to various PTC concentrations mixed in the drinking water (PTC-solution). In trials 1 and 2 (n = 15/trial), 0.20, 0.56, 1.57, 4.39, and 12.29 mM and 0.20, 0.43, 0.94, 2.03, and 4.39 mM of PTC were tested, respectively. On test days, PTC-solution (trial 1: 1.5 kg; trial 2: 3.0 kg) and water (same amounts) were offered for *ad libitum* intake in a side-by-side presentation for 1 h in trial 1 and 2 h in trial 2. Each test day was followed by a rest day where PTC-solution was replaced with water to limit potential carry over effects into the next test day. Consumption of PTC-solution for each PTC concentration was expressed as the percentage of PTC-solution intake of total morning fluid intake. There was no effect ($P > 0.74$) of sequence that rams received PTC-solutions on PTC consumption during either trial. As PTC concentration increased, percentage of PTC-solution intake decreased ($P \leq 0.01$) for both trials. The greatest decrease in percentage of PTC-solution intake occurred between 1.57 – 4.39 mM (58%) for trial 1 and 2.03 – 4.39 mM (72%) for trial 2. In trial 2, the least percentage of PTC-solution intake was the 4.39 mM PTC concentration, which was different ($P \leq 0.05$) from lesser PTC concentrations.

All other PTC concentrations did not differ ($P > 0.05$) from each other in percentage intake. This research suggests rams could taste the PTC, and the concentration at which PTC-solution was avoided varied across rams. It may be possible to select sheep, based on demonstrated avoidance of PTC, for targeted grazing applications to manipulate vegetation towards range management goals.

Key words: bitterness avoidance, phenylthiocarbamide, sagebrush, sheep

Introduction

Overgrowth of mountain big sagebrush (*Artemisia tridentate* Nutt. ssp. *vaseyana*) can lead to a reduction in rangeland plant diversity, carrying capacity, and wildlife abundance (Johnson et al., 1996; Launchbaugh, 2003). Sagebrush can be controlled or eliminated by plowing, burning, and spraying (Wambolt and Payne, 1986), but these methods can be expensive and have potential undesirable effects on rangelands. One method of control that has received minimal attention, and may be more sustainable, is reduction of sagebrush with grazing sheep. Using fecal analysis over several experiments, Snowder et al., (2001) indicated that the dietary preference for sagebrush in sheep has a heritability of 0.28, suggesting that selection against bitterness avoidance in sheep breeding programs may be feasible. Furthermore, Ferreira et al. (2013) identified a set of novel genes for bitter taste receptors in sheep, suggesting that sheep may be genetically predisposed to select or avoid plants with bitter or noxious tastes.

Sheep are adaptive selective grazers (Launchbaugh et al., 2001) with varying dietary preference for consuming sagebrush (Bork et al., 1998; Snowder et al., 2001; Seefeldt, 2005). Several factors can be attributed to an individual's diet preference/selection including learned behaviors, taste preference, post digestive feedback, and their ability to detoxify secondary metabolites. Many toxic forages have a bitter taste, but the toxicity and the correlation of bitter taste to toxicity varies (Cedarleaf et al., 1983; Johnson et al., 1985). Avoidance to bitter tasting plants is a mechanism sheep utilize to limit toxin ingestion (Launchbaugh et al., 2001). Early research on the primary taste groups of sweet, sour, salty and bitter in sheep suggested that bitterness maybe the most sensitive (Goatcher

and Church, 1970a). Additional studies indicated that sheep can taste and(or) sense bitterness when mimicked by addition of compounds, like quinine, when added to drinking water (Goatcher and Church, 1970a; Favreau et al., 2010), and lithium chloride, when added to forages (Launchbaugh and Provenza, 1994).

Phenylthiocarbamide (PTC) is a compound, not found in nature, that mimics bitter tastes found in food (Blakeslee and Salmon, 1935; Barnicot et al., 1951; Lee and O'Mahony, 1998), and has been used in bitter taste research in humans (Blakeslee, 1932; Fox, 1932; Harris and Kalmus, 1949) and mice (Lush, 1986; Nelson et al., 2003). In humans, PTC thresholds have been suggested to be heritable ($h^2 = 0.55$) (Morton et al., 1981). It has also been suggested that PTC avoidance is influenced by post-digestive factors (Nelson et al., 2003), similar to the preferences of sheep grazing bitter/toxic forages (Launchbaugh et al., 2001).

This study focused on bitter taste avoidance (Parker, 2003) by the addition of PTC in water. We hypothesized that sheep could detect bitter-tasting compounds and the sensitivity would vary from animal to animal. The objective of this study was to determine whether sheep could detect the bitter tasting compound, phenylthiocarbamide, and if so, what PTC concentration would elicit an avoidance response.

Methods and Materials

Animals

All animal procedures were approved by an Institutional Animal Care and Use Committee (USDA, ARS, Dubois, ID) in accordance with the USDA, APHIS Animal Welfare Regulations (2013; 9 C.F.R. § 2.30-2.38 2013) and the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Two trials were conducted at the USDA, ARS U.S. Sheep Experiment Station located near Dubois, Idaho in the spring of 2018. In trial 1, yearling Rambouillet ($n = 7$) and Targhee ($n = 8$) rams (initial BW = 76.6 ± 5.7 and 83.7 ± 9.1 kg, respectively) were used; while in trial 2, yearling Rambouillet ($n = 6$) and Targhee ($n = 9$) rams (initial BW = 83.0 ± 9.7 and

93.5 ± 9.14 kg, respectively) were used. For the duration of both trials, rams were housed indoors in individual pens, so feed and water intake could be monitored under controlled conditions of 10°C with a 12 h light:dark cycle. Additionally, feed and water were withheld from rams from 1700 to 0700 h each day during the trials. For each trial, rams were randomly allotted within breed to alternate pens throughout the barn. Both trials were divided into two phases; an acclimation phase, where rams were adjusted to the pens and daily feed and fluid delivery routines, and a testing period, where the phenylthiocarbamide (PTC) treatments were delivered.

Experimental design

Both trials were conducted as a cross-over design consisting of 5 PTC treatments with individual rams receiving a different PTC concentration each test day. In order for all rams to be tested in a day, rams were randomized to five testing blocks consisting of three rams each. Each block was randomly assigned a PTC testing sequence, which consisted of the order in which rams received their PTC treatments over the five test days (Table 2.1).

Each trial consisted of a 5-d acclimation phase followed by the PTC testing. During the acclimation phase of both trials, rams received alfalfa pellets (Table 2.2) at a rate of 1.9% of BW (as fed basis) at 0700 h. Thirty minutes after feeding, feed was removed, and refusals weighed. Immediately following feed removal, two buckets filled with water (1.5kg, trial 1; 3.0 kg, trial 2) were placed in each pen and rams were allowed access to the water for 1 h in trial 1 and 2 h in trial 2. Buckets were placed side-by-side in a holding rack and given a designation of left and right side. At the end of the first water consumption period, buckets were removed from each pen and water refusals weighed for each bucket and discarded. After removal of buckets, pens were cleaned, and rams were given their daily feed and water at approximately 0900 h for trial 1 and 1000 h for trial 2. In trial 1, rams received alfalfa pellets (Table 2.2) fed at a rate of 2.8% of BW (as fed basis), 45 g of a mineral mix (Table 2.2), and 5 kg of water in a single bucket. At approximately 1230 h, the water bucket was removed, refusals weighed, and discarded. An additional 4 kg of water was offered, and at

1700 h, all feed and water were removed, refusals weighed, and discarded. Whereas in trial 2, rams received alfalfa pellets fed at a rate of 2.8% of BW (as fed basis), 45 g of a mineral mix, and two buckets with each containing 4 kg of water were offered, and at 1700 h, all feed and water were removed, refusals weighed, and discarded.

The PTC was chosen as a bitter tasting agent to mimic the attributes of monoterpenoids, which are often found in toxic shrubs. It is unknown what PTC concentration mimics the degree of bitterness in plants; therefore, PTC concentrations for trial 1 were chosen over a large range, then adjusted for trial 2 to better meet the objectives of the study. In trial 1 some individuals consumed all fluid in either bucket offered during the testing times. Therefore, in order to limit thirst as a potential factor in consumption, the volume of water and PTC-solution offered were increased while the time allotted for consumption was also increased for trial 2.

The test phase for both trials consisted of test days where PTC solutions and water (in separate buckets) were delivered after the morning feeding, and each test day was followed by a rest day where only water was delivered in order to minimize potential carry-over effects of PTC from the previous test day. Tap water **(water)** from the Sheep Experiment Station well was used for this study. For test and rest days, the same procedures relative to timing of feed delivery, number of buckets, and total amounts of fluid delivered were followed as per the acclimation phase. On a test day, each ram block received one of the five concentrations of PTC-solutions (trial 1: 0.20, 0.56, 1.57, 4.39, or 12.29 mM delivered in a total volume of 1.5 kg; trial 2: 0.20, 0.43, 0.94, 2.03, or 4.39 mM delivered in a total volume of 3.0 kg) in one bucket, and water only (trial 1: 1.5 kg; trial 2: 3.0 kg) in the other bucket. The location (left or right) of the PTC-solution bucket was alternated between test days. On the subsequent rest day, no PTC-solution was administered and was replaced with water. For both trials, PTC (Sigma P7629, Sigma-Aldrich, Sant Louis. MO) was dissolved in absolute ethanol then diluted with water to the desired concentrations for delivery.

Statistical Analysis

For all fluid intake variables analyzed within a trial, data were analyzed using PROC MIXED procedures of SAS (Statistical Analysis System, SAS Institute Inc., Version 9.4, Cary, NC). The model included treatment (PTC concentration), sequence (order PTC concentrations were administered to rams), and period (day that PTC was administered within the sequence) with a random statement that included ram within sequence. Means are reported as least squares means, and mean comparisons were made using pair-wise contrasts (PDIFF). Significance was set at $P \leq 0.05$.

Due to the variation in avoidance to PTC observed within and across PTC concentrations for each trial (Table 2.3), individual rams were further classified into consumer groups based upon total PTC intake (g) over the five test days. Consumer group differentiation was determined by 0.5 standard deviation of the population mean to divide rams into high (≥ 0.5 SD), medium (< 0.5 to > -0.5 SD), or low (≤ -0.5 SD) PTC consumers (Table 2.4). One objective of this study was to evaluate variation among individual rams. To test the variation observed, linear regression using PROC GLM for analysis by consumer group with the independent variable being PTC concentration and dependent variable included percent of PTC-solution intake of test fluid intake. Orthogonal and paired contrasts were used to test coincidence of regression lines (slope and intercept analyzed together), as well as slopes, and intercepts individually between PTC consumption groups.

Results and Discussion

We hypothesized sheep could detect PTC when mixed in water and that sensitivity would be different among individuals. Unlike in human studies (Blakeslee and Salmon 1935, Fox 1932), rams are unable to verbally express if they can detect PTC. Although behavioral data was not quantified in this experiment, PTC concentrations where the PTC-solution was consumed less than water negative behavioral reactions were observed during the study (e.g. smacking lips and shaking their head after tasting the PTC), particularly with the highest PTC concentrations (data not shown). Furthermore, as PTC concentration increased, mean intake of the PTC-solutions decreased (Tables 2.5 and 2.6).

Individual reactions and intake of PTC-solution, taken altogether, suggest that rams could detect PTC, and that animals varied in sensitivity to detection of PTC.

Consumption of PTC-solution expressed as a percentage of total morning fluid intake is depicted in Tables 5 and 6 (Trials 1 and 2, respectively). In trial 1, as each solution increased in PTC concentration the intake of PTC-solution decreased ($P < 0.001$). The greatest decrease in percentage of PTC-solution intake was observed between 1.57 – 4.39 mM (58%) in trial 1. As PTC concentration increased, PTC-solution intake decreased, and water intake increased ($P < 0.001$).

In trial 2, the greatest decrease in percentage of PTC-solution intake was observed between 2.03 – 4.39 mM (72%). There was also a treatment effect ($P < 0.01$) on PTC-solution intake but a slightly different trend was observed than in trial 1. The intake of the 0.20, 0.43, 0.94, and 2.03 concentrations were all similar ($P > 0.05$), but the intake of the 4.39 mM concentration was different ($P \leq 0.05$) than the rest. Similar to observations from trial 1, intake of the water increased as PTC increased in trial 2. The limited dose response in trial 2 may be due to the smaller differences between PTC concentration levels for that trial and/or the increase in total morning fluid offered. Trial 1 PTC concentrations were chosen at approximately multiples of three; whereas in trial 2 PTC concentration were chosen at approximately multiples of two. Trial 1 and trial 2 results showed that the greatest decrease in PTC-solution consumption occurred when PTC concentrations increased from 1.57 mM – 4.39 mM, which suggests a threshold within this range may have possible implications in determining bitter taste avoidance in sheep.

The lowest PTC concentration (0.20 mM) for both trials had lower intake than the water. This observation suggests PTC is detectable and avoidance begins for some rams below 0.20 mM. This is supported by the observation that minimum consumption of the 0.20 mM PTC-solution were 0.5 and 0.3% of total fluid intake for trials 1 and 2, respectively (Table 2.3). It should also be noted, however, that within the 0.20 mM PTC concentration that maximum PTC-solution consumption was > 95% for trials 1 and 2 (Table 2.3). In trial 2, the mean consumption of the 0.20 mM PTC concentration is

approximately half of that observed in trial 1. This difference could be attributed to the amount of time allotted to consume test fluid. If a ram decided to avoid a particular PTC concentration, there would be more time in trial 2 to consume water post avoidance decision.

Nelson et al. (2003) observed a similar inverse relationship between PTC concentration and average intake when PTC-solutions were administered to mice. Similarly, Church and Goatcher (1970a) administered increasing concentrations of quinine (a bitter-mimicking agent) in drinking water to rams alongside a control of water and observed an inverse relationship between concentration of quinine and percent of quinine solution of fluid intake. Church and Goatcher (1970b) further studied the sensitivity to quinine in a subsequent study, and when analyzed on an individual basis. Similar to this study, considerable variation from the mean was observed in percentage of solution intake of test fluid intake for each concentration. This large degree of difference in sensitivity among individuals has also been observed in human research and has led to the categorization of individuals into tasters, and non-tasters (Fox, 1932; Blakeslee and Salmon, 1935). Research in humans has typically placed participants into upper or lower thresholds to categorize tasters, non-tasters, and super tasters, which was originally suggested by Bartoshuk et al. (1994). Tasters are categorized as ‘tasters’ if they can detect PTC at a low concentration and as ‘non-tasters’ when detection is not until they consume a high concentration (Blakeslee and Salmon, 1935; Harris and Kalmus, 1949). The lack of standardization of testing sensitivity to PTC has produced inconsistent conclusions (Tepper, 2008). In this study, some individuals (tasters) consumed less than 1% of the 0.20 mM PTC concentration, and other individuals (non-tasters) consumed >95% in both trials. Including the observations made by Church and Goatcher (1970a; 1970b) and those from this study, sheep might fall into similar categories as humans.

While it is known that sheep will tolerate bitter-tasting compounds (Provenza et al., 1992; Launchbaugh et al., 2001), there is no previous literature indicating PTC tolerance thresholds in sheep. In human research, PTC categories have been suggested to be associated with bitterness

intensity perception (Blakeslee and Salmon, 1935; Bartoshuk et al., 1994; Drewnowski and Rock, 1995). However, quinine sensitivity and PTC sensitivity in humans are variable where some individuals perceive quinine as being more bitter than PTC and some individuals perceive PTC as being more bitter than quinine (Blakeslee and Salmon, 1935; Frank and Korchmar, 1985). The bitter tasting compound PTC contains a thiocyanate moiety (Bartoshuk et al., 1994), which is similar to isothiocyanates. Isothiocyanates are produced during the breakdown of glucosinolates, commonly found in bitter tasting vegetables (Ettlinger and Lundeen, 1957). Quinine and PTC both elicit bitter tastes, but likely due to its' chemical makeup, PTC sensitivity has been linked to glucosinolates preference (Duffy and Bartoshuk, 2000). Church and Goatcher (1970a) observed a similar inverse relationship between increasing quinine solution concentrations and decrease in consumption as described in this study, but sensitivity to quinine or PTC may translate differently to foraging preferences in sheep.

For both trials, there were no sequence effects ($P > 0.05$) observed for percentage of PTC-solution consumed, indicating that the sequence in which rams received the PTC solutions did not affect fluid intakes on subsequent test days (Tables 2.4 and 2.5). There was also no sequence effect ($P > 0.05$) on total fluid intake on rest days, which suggested the effects of PTC dissipate rapidly (data not shown). Relative to both PTC treatment and sequence that rams received it on a test day, there were no treatment or sequence effects ($P > 0.05$) on the amount of water intake during the rest days for the morning, afternoon, and total fluid intake. The average percentage of water intake on the rest days during the morning, afternoon, and total for the day were 56.8 ± 7.7, 56.8 ± 3.5 and 82.6 ± 3.0 %, respectively for trial 1 and 76.6 ± 4.7, 90.1 ± 3.0, and 84.3 ± 2.3 %, respectively for trial 2.

A great deal of variation in PTC-solution intake was observed between rams (Table 2.4). In trial 1, the ram with the greatest intake of PTC consumed 9.7-fold more PTC than the ram with the lowest intake (1.06 vs. 0.109 g, respectively). For trial 2, the magnitude of difference was much greater at 60-fold (2.10 vs. 0.0348 g PTC, respectively). Based on the variation between rams within

each trial, rams were grouped according to total (g) PTC intake (Table 2.4). In trial 1, the high intake group consisted of three individuals, medium consisted of five, and low consisted of seven, where in trial 2, all groups consisted of five individuals.

Similar to the sensitivity observed in this study, sensitivity to consuming bitter shrubs has also been observed in grazing sheep. Snowder at el. (2001) determined percentage of sagebrush consumed in the diet of 549 ewes was 10.3 – 31.9% for September and 23.7 – 42.3% for October. The September and October measurements were highly correlated ($r_2 = 0.91$), where the highest consumers in September were also the highest consumers in October, similar to this study, where the individuals in the high consumer group consistently consumed the most PTC-solution.

Variation was also observed in total daily fluid intake among the rams in this study. Based on this observation, to account for individual total fluid intake variation, we used the percent of PTC-solution intake of total morning fluid intake (Figures 2.1 and 2.2, and Tables 2.5 and 2.6). Regression analyses were performed on each consumer group within each trial based on percentage of PTC-solution intake of total morning fluid intake (Figures 2.1 and 2.2). The slopes of each consumer group within each trial did not differ ($P > 0.05$), suggesting that the rate of avoidance between consumer groups was not different. However, most of the intercepts differed across consumer groups (Figures 2.1 and 2.2), which suggests that the point of avoidance as PTC concentration increases is different between groups.

In trial 1, within the medium and low groups, no individual consumed more than 5% of the highest PTC concentration (12.29 mM) offered. Because the point at which the greatest avoidance within the population was observed between the 1.57- and 4.39-mM PTC concentrations in trial 1, the 12.29 mM concentration was eliminated for trial 2. Furthermore, because the range of PTC concentrations for trial 2 was smaller than that of trial 1, the amount of PTC-solution and water offered in the morning and the time allotted for intake were increased. These changes made from trial 1 to trial 2 were to encourage those individuals that were willing to consume greater concentrations of

PTC to differentiate themselves from the population. Although PTC concentrations, total fluid offered, and duration that the PTC-solution was available to the rams varied between trials, a similar individual variation in PTC-solution intake was still observed in trial 2 compared with trial 1 (Figures 2.1 and 2.2). These results suggest that PTC intake is related to the individual ram's preference for, or avoidance to, bitter taste and that PTC can be used as a bitter-mimicking agent to determine sensitivity to bitterness among individuals.

To date, this is the first study to use PTC to test for sensitivity of bitterness among sheep. Sheep have displayed the ability to identify the presence of terpenes when fed in a mixed ration (Villalba et al., 2006; Mote et al., 2007), which suggests that part of diet selection is sensory and is related to taste and(or) aroma. Dziba and Provenza (2008) reported that in lambs offered varying concentrations of monoterpenoids (camphor, p-cymeme,1-8 cineole, methacrolein; commonly found in Big Mountain sagebrush) mixed into their diets, intake rates of the mixed diet in relation to monoterpene concentration varied. There was no difference in the percentage of time spent eating among the groups (high concentration = 4.65% terpene, medium concentration = 3.10%, and low concentration = 1.55%), but the medium group consumed less than the low group and the high group consumed less than the medium group. Furthermore, Dziba and Provenza (2008) suggested that lambs regulate feed intake of bitter vegetation to prevent consuming a toxic dose of terpenes. Although total amount of forages consumed differed between the medium and high group, both groups stopped consuming feed when they reached approximately 28 g of monoterpenoids per day (Dziba and Provenza, 2008). Launchbaugh et al. (1993) observed similar behavior when lithium chloride was mixed in diets fed to lambs. Despite the concentration at which lithium chloride was fed, lambs regulated their total intake to not exceed concentrations of 62.7 ± 4.5 mg/kg lithium chloride per day. Regulating intake of bitter-tasting compounds is likely a developed mechanism that sheep use to avoid forages that have negative post-ingestion qualities (Provenza and Balph, 1987). While post-

ingestion feedback is one mechanism used by ruminants in forage selection, it is likely not the only deciding factor.

Launchbaugh (2001) suggested that foraging behaviors can be learned from mimicking maternal and herd behavior, and taste memory from suckling. Nolte and Provenza (1992) observed that feeding onion-flavored milk to orphan lambs resulted in a preference for onion-flavored feeds later in life. Some literature suggests that bitterness is likely not the apparent causative factor when consuming toxic forages, but rather post-ingestive feedback mechanisms (Provenza et al., 1992; Launchbaugh et al., 2001). Future selection or determent of a forage is associated with the memory of that taste and the digestive feedback; however, memory and tolerance vary between individuals and each individual perceives cost/benefit from a forage differently (Sclafani, 1991; Provenza et al., 1992; Launchbaugh et al., 2001). Differences in memory of a forage is likely linked to the individual's physiologic ability to suppress the toxic effects (Provenza et al., 1992). Because terpenoids contain bitter-tasting compounds, variation in bitter preference between individuals may not only translate to forage selection but may also be correlated with the individual's ability to suppress toxins. Toxic shrub intake is likely driven by several phenotypic (Dziba et al., 2007; Ginane et al., 2011; Mennella et al., 2005) and genotypic factors (Chandrashekar et al., 2000; Bufe et al., 2005).

Results from this study indicate that there are sensitivity differences between individual's preference for consuming bitter tasting compounds in sheep. The variation in bitterness intake may translate to foraging preferences while grazing, where rams with greater tolerance for bitter taste may consume plants with higher concentrations of bitter tasting compounds, such as monoterpenoids. Similarly, humans that are categorized as non-tasters consume more anti-oxidant rich vegetables with bitter attributes than tasters (Garcia-Bailo et al., 2009).

Utilizing sheep as a grazing tool to reduce sagebrush canopy has been suggested to entail long-term and high-intensity grazing applications (Seefeldt, 2005); however, sheep grazing may be a good tool for suppressing sagebrush canopy growth and decrease shrub encroachment on grasslands.

Moffet at el. (2015) suggested that during a rangeland life cycle in a mountain big sagebrush ecosystem, the greatest forage productivity and optimal wildlife habitat conditions occur 5-15 years post-fire. Furthermore, productivity of rangeland decreases as sagebrush canopies become overgrown. Johnson at el. (1996) suggested that the greatest ecological diversity in mountain big sagebrush ecosystems occurs when the sagebrush canopy makes up approximately 15% of total plant composition, and the greatest herbaceous production occurs when the sagebrush canopy makes up 11-17% of total plant composition. Because diet selection is moderately heritable in sheep ($h^2 = 0.28$) (Snowder et al., 2001), selection for sheep that have a higher tolerance for bitter tasting compounds may translate to sagebrush canopy growth suppression on rangeland, and therefore, extend the optimal ecological productivity-time period beyond 5-15 years post-fire (Moffet et al. 2015).

Acknowledgments

The authors would like to acknowledge the support for this research provided by the USDA-ARS-Sheep Experiment Station employees, Mark Williams, Taylor Hudson, and Ashleigh Redman. The authors would also like to thank Dr. William Price, University of Idaho, College of Agriculture and Life Sciences for his assistance with the statistical analysis.

Tables and Figures

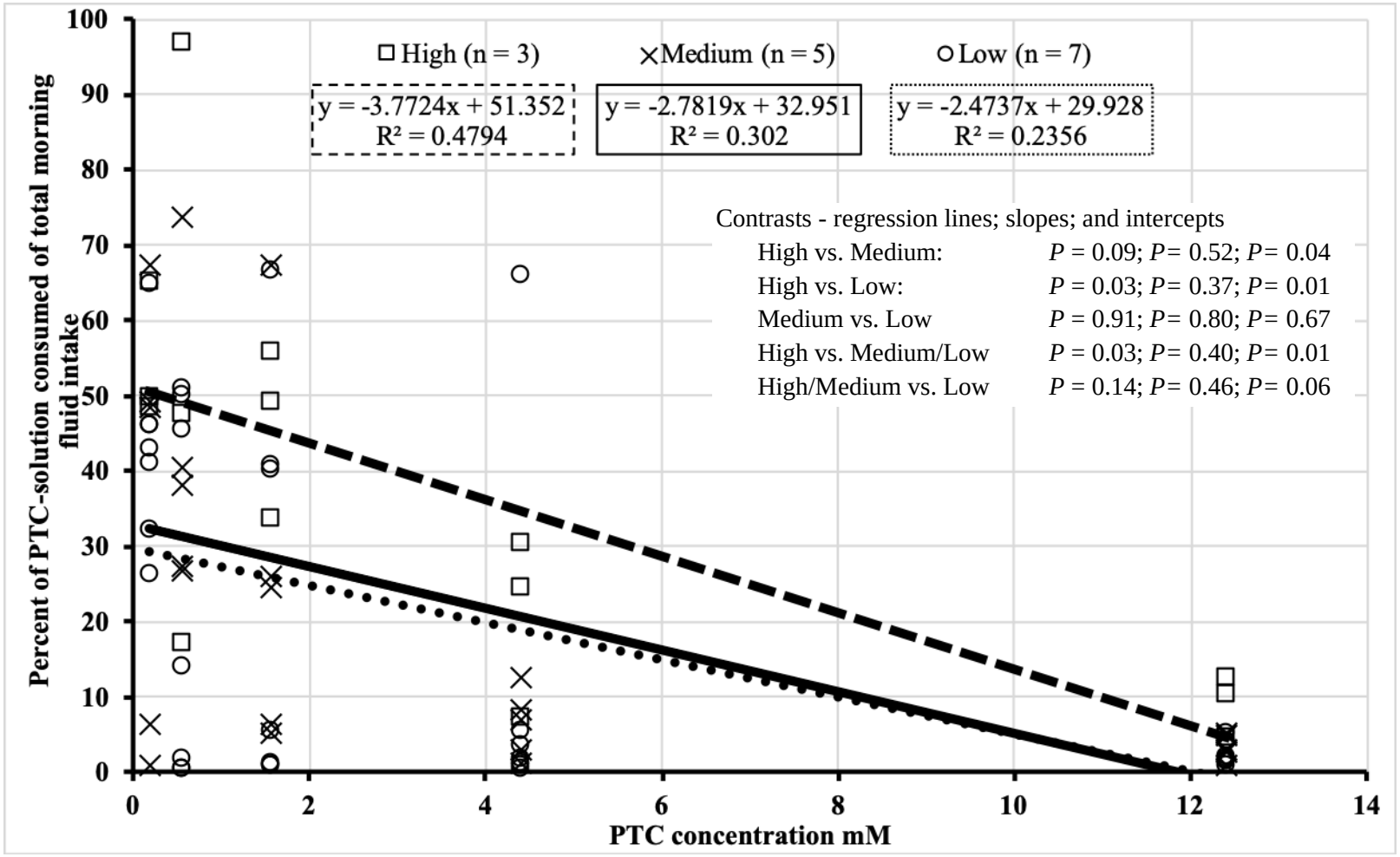


Figure 2.1 Trial 1 linear regressions of phenylthiocarbamide (PTC) consumption by total PTC intake categories (High: □ – – –; Medium: × ——; Low: ○ • • • •) for Rambouillet and Targhee rams administered five PTC concentrations (0.20, 0.56, 1.57, 4.39, and 12.29 mM) suspended in 1.5 kg of water. Paired contrast made between High vs Low, Medium vs Low and Low vs High. Orthogonal contrast made between High/Medium vs Low and High vs Medium/Low.

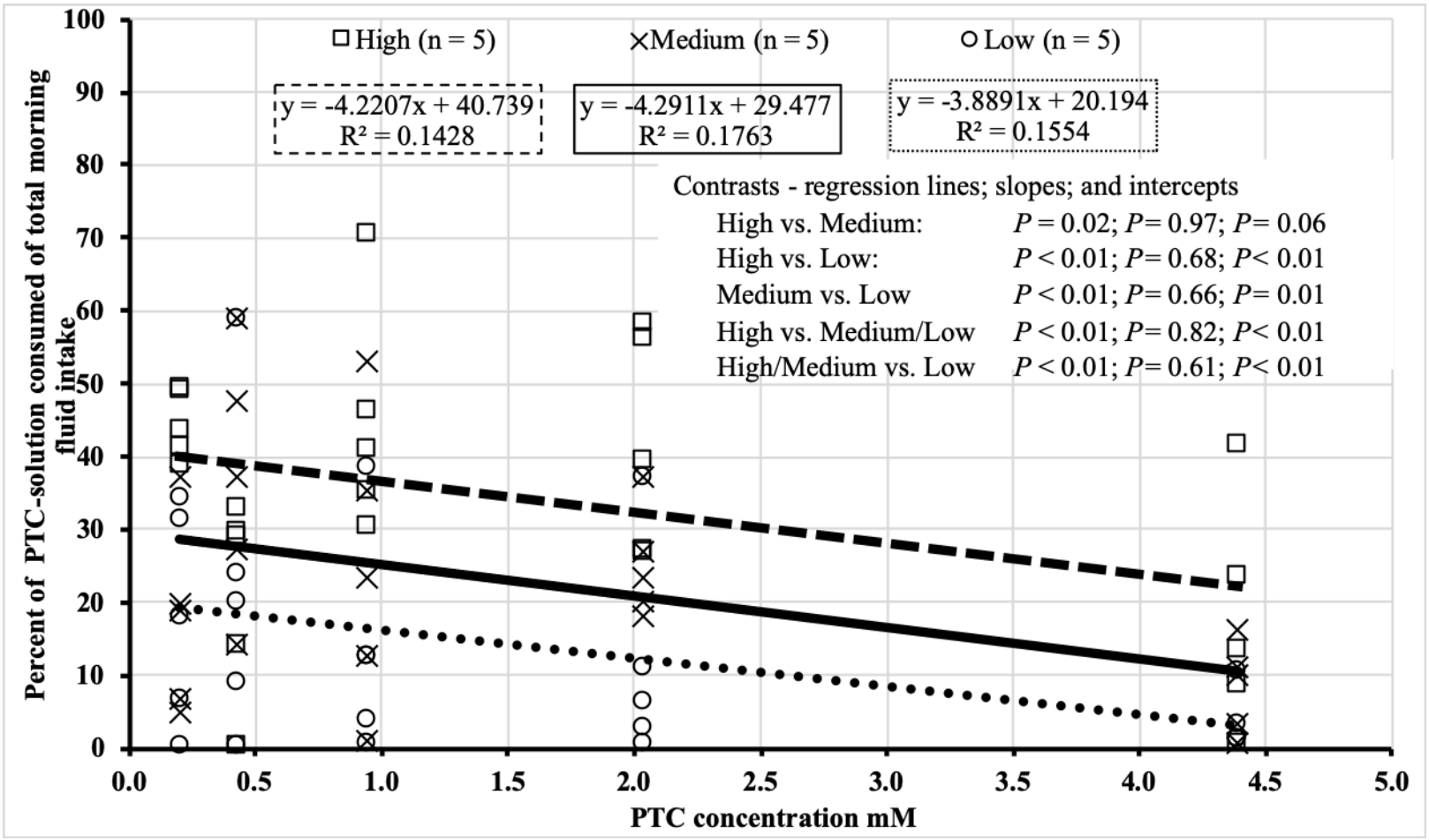


Figure 2.2. Trial 2 linear regressions of phenylthiocarbamide (PTC) consumption by total PTC intake categories (High: □ – – –; Medium: × ——; Low: ○ • • • •) for Rambouillet and Targhee rams administered five PTC concentrations (0.20, 0.43, 0.94, 2.03, and 4.39 mM) suspended in 3.0 kg of water. Paired contrast made between High vs Low, Medium vs Low and Low vs High. Orthogonal contrast made between High/Medium vs Low and High vs Medium/Low.

Table 2.1 Sequence in which blocks of rams received each PTC-solution concentration for both trials. Each group consisted of 3 rams with a total of 15 rams tested on each test day per trial.

Test Day	**Trial 1 - PTC Concentrations (mM)**				
	0.20	**0.56**	**1.57**	**4.39**	**12.29**
1	Group 1	Group 2	Group 3	Group 4	Group 5
2	Group 2	Group 5	Group 4	Group 1	Group 3
3	Group 5	Group 3	Group 1	Group 2	Group 4
4	Group 3	Group 4	Group 2	Group 5	Group 1
5	Group 4	Group 1	Group 5	Group 3	Group 2
	Trial 2 - PTC Concentrations (mM)				
	0.20	**0.43**	**0.94**	**2.03**	**4.39**
1	Group 1	Group 2	Group 3	Group 4	Group 5
2	Group 2	Group 5	Group 4	Group 1	Group 3
3	Group 5	Group 3	Group 1	Group 2	Group 4
4	Group 3	Group 4	Group 2	Group 5	Group 1
5	Group 4	Group 1	Group 5	Group 3	Group 2

Table 2.2. Sequence in which blocks of rams received each PTC-solution concentration for both trials.

	PTC-solutions				
	A (lowest)	**B**	**C**	**D**	**E (highest)**
Day 1 – TEST	**Group 1 (n = 3)**	**Group 2 (n = 3)**	**Group 3 (n = 3)**	**Group 4 (n = 3)**	**Group 5 (n = 3)**
Day 2 – REST					
Day 3 – TEST	**Group 2 (n = 3)**	**Group 5 (n = 3)**	**Group 4 (n = 3)**	**Group 1 (n = 3)**	**Group 3 (n = 3)**
Day 4 – REST					
Day 5 – TEST	**Group 5 (n = 3)**	**Group 3 (n = 3)**	**Group 1 (n = 3)**	**Group 2 (n = 3)**	**Group 4 (n = 3)**
Day 6 – REST					
Day 7 – TEST	**Group 3 (n = 3)**	**Group 4 (n = 3)**	**Group 2 (n = 3)**	**Group 5 (n = 3)**	**Group 1 (n = 3)**
Day 8 – REST					
Day 9 – TEST	**Group 4 (n = 3)**	**Group 1 (n = 3)**	**Group 5 (n = 3)**	**Group 3 (n = 3)**	**Group 2 (n = 3)**

Table 2.3. Alfalfa pellets and mineral supplement component analysis (DM basis)

Item	**Alfalfa pellets**[1]	**Mineral supplement**[2]
Dry matter, %	100	100
Crude protein, %	17.4	-
Acid detergent fiber, %	36.8	-
Total digestible nutrients	54.8	-
Ca, %	1.79	0.85
P, %	0.22	0.002
K, %	2.09	0.03
Mg, %	0.29	0.06
S, %	0.28	0.07
Na, %	0.16	95.0
Zn, mg/kg	22.6	1
Fe, mg/kg	717	300
Mn, mg/kg	50	5
Cu, mg/kg	7.8	3
Mo, mg/kg	2.17	-

[1]Component analysis of alfalfa pellets conducted by Ward Laboratories (Kearney, NE).
[2]Mineral supplement formulated by Redmond Agriculture (Redmond, UT). Product name “10 Fine Premium Mineral Salt”.

Table 2.4. Descriptive statistics of variation observed across phenylthiocarbamide (PTC) concentration categories within each trial where values are represented as percentage of PTC-solution intake of total fluid offered and percentage of water intake of total fluid offered. All units are expressed as percentages.

	Trial 1							
PTC concentration, mM	**Mean** ± SD		**Minimum**		**Maximum**		**Coefficient of variation**	
	PTC	**Water**	**PTC**	**Water**	**PTC**	**Water**	**PTC**	**Water**
0.20	54.5 ± 36.0	67.3 ± 35.9	0.5	1.0	95.7	100	66.1	53.3
0.56	39.0 ± 33.5	65.0 ± 35.8	0.4	1.0	93.1	100	86.0	55.0
1.57	30.9 ± 32.4	71.5 ± 32.5	0.5	8.2	94.7	100	105.0	45.4
4.39	7.2 ± 9.1	81.2 ± 26.9	0.3	0.6	32.2	100	126.7	33.1
12.29	3.6 ± 4.0	77.7 ± 26.0	0.7	25.9	14.3	100	112.1	33.5
	Trial 2							
0.20	41.9 ± 33.8	92.7 ± 10.1	0.3	69.1	97.6	100	80.8	10.9
0.43	29.9 ± 23.9	88.0 ± 22.0	0.3	28.6	90.9	100	79.7	25.0
0.94	40.5 ± 34.5	88.0 ± 18.3	0.5	39.6	95.8	100	85.1	20.8
2.03	33.1 ± 27.9	89.5 ± 17.3	0.3	53.0	89.8	100	84.3	19.3
4.39	11.5 ± 15.4	90.2 ± 16.7	0.5	49.2	57.1	100	133.3	18.5

Table 2.5. Descriptive statistics of phenylthiocarbamide (PTC) consumption categories based on rankings of total PTC consumption by individual rams across the five test days within a trial. Thresholds determined by mean ± (0.5 × SD).

Trial 1			Trial 2		
Ram ID	**Consumption group**	**Total PTC intake (g)**	**Ram ID**	**Consumption group**	**Total PTC intake (g)**
T6581	High	1.056	T6886	High	2.103
S0791	High	0.876	T6342	High	1.389
S1497	High	0.769	S0801	High	1.318
S0583	Medium	0.408	T6884	High	1.236
T6406	Medium	0.384	T6093	High	1.163
S1500	Medium	0.305	T6516	Medium	0.932
T6885	Medium	0.294	T6313	Medium	0.781
T6578	Medium	0.281	S1499	Medium	0.737
T6502	Low	0.207	T6582	Medium	0.711
T6883	Low	0.205	T6299	Medium	0.697
T6297	Low	0.204	S1069	Low	0.452
S1501	Low	0.166	S0912	Low	0.327
S1125	Low	0.106	S1498	Low	0.177
S1124	Low	0.104	T6580	Low	0.091
T6401	Low	0.082	S1126	Low	0.035
Mean ± SD for all rams		0.363 ± 0.299			0.810 ± 0.567

Table 2.6. Mean fluid intakes of rams receiving either water or phenylthiocarbamide (PTC) during a test period for Trial 1

	PTC concentration, mM						*P-values*		
Variable	**0.20**	**0.56**	**1.57**	**4.39**	**12.29**	**Pooled SE**	**Treatment**	**Sequence**	**Period**
Total test fluid intake as a percentage of total offered (1.5 kg water and 1.5 kg PTC-solution)	60.9$_{a}$	52.0$_{a, b}$	51.2$_{a, b}$	44.2$_{b}$	40.7$_{b}$	6.3	0.02	0.74	0.33
Water intake as a percentage of total test fluid intake	57.6$_{a}$	64.5$_{a, b}$	72.1$_{b}$	88.4$_{c}$	95.9$_{c}$	5.0	0.0001	0.39	0.11
PTC-solution intake as a percentage of total test fluid intake	42.4$_{a}$	35.5$_{a, b}$	27.9$_{b}$	11.6$_{c}$	4.1$_{c}$	5.0	0.0001	0.39	0.11
Afternoon water intake as a percentage of total offered (9 kg)	92.5	91.4	91.6	89.5	92.9	3.2	0.61	0.68	0.03
Total fluid intake as a percentage of total fluid offered (12 kg)	84.6$_{a}$	81.5$_{b}$	81.5$_{b}$	78.2$_{c}$	79.9$_{b,c}$	2.6	0.003	0.54	0.002

$_{a,b,c}$ Means with different superscripts within a response and across PTC concentrations are different ($P \leq 0.05$).
Treatment refers to PTC concentrations, sequence is the order PTC concentrations that were administered to rams, and period is the day PTC was administered within the sequence.

Table 2.7. Mean fluid intakes of rams receiving either water or phenylthiocarbamide (PTC) during a test period for Trial 2

	PTC concentration, mM						*P-values*		
Variable	**0.20**	**0.43**	**0.94**	**2.03**	**4.39**	**Pooled SE**	**Treatment**	**Sequence**	**Period**
Total test fluid intake as a percentage of total offered (1.5 kg water and 1.5 kg PTC-solution)	67.3$_{a}$	58.9$_{b}$	64.3$_{a, b}$	61.3$_{a,b}$	50.8$_{c}$	4.5	0.0002	0.86	0.02
Water intake as a percentage of total test fluid intake	73.6$_{a}$	75.9$_{a}$	72.9$_{a}$	76.3$_{a}$	90.3$_{b}$	4.8	0.01	0.98	0.87
PTC-solution intake as a percentage of total test fluid intake	26.4$_{a}$	24.1$_{a}$	27.1$_{a}$	23.7$_{a}$	9.7$_{b}$	4.8	0.01	0.98	0.87
Afternoon water intake as a percentage of total offered (9 kg)	86.2	91.7	94.1	95.6	95.9	2.7	0.06	0.07	0.01
Total fluid intake as a percentage of total fluid offered (12 kg)	78.1	77.7	81.3	80.9	76.6	2.5	0.25	0.46	0.005

$_{a,b}$ Means with different superscripts within a variable and across PTC concentrations are different ($P \leq 0.05$).
Treatment refers to PTC concentrations, sequence is the order PTC concentrations were administered to rams, and period is the day PTC was administered within the sequence.

Chapter 3: Comparative Genomics of the Sheep Tas2r Repertoire to Cattle, Goat, Human, Dog and Mice

Abstract

Type two taste receptors (Tas2r) are the only taste receptors that distinguish bitter-tasting compounds. Human Tas2r genes have been extensively studied and have been associated with dietary preferences, health, substance dependence, and other diseases. Sheep are an important livestock species known for grazing vast rangelands with variable ecology and plant communities. However, the limited work related to Tas2r gene repertoires in the reference genomes of grazing animals creates a challenge for understanding how these genes influence diet selection preferences. Tas2r genes typically cluster on two regions of the genome. In the second cluster of the sheep (OAR_rambouillet_1.0), goat (ARS1), and cattle (ARS-UCD1.2) reference genomes, there are six, nine, and two Tas2r genes that were not annotated, respectively. Comparative genomic strategies were used to cross-reference sheep Tas2r genes cattle, goat, human, dog, and mice for the proposed annotation. A nucleotide similarity comparison of the whole Tas2r repertoires for the three grazing species suggested that goat and cattle are similar to sheep (≥ 95.5% and ≥ 91.9% similarity, respectively). Several Tas2r genes found in sheep, cattle, and goat are likely not found in human, dog, or mice and may be reserved to ruminants or animals of similar feeding ecology. Using a comparative genomics approach, this paper proposes annotations for sheep, cattle, and goat Tas2r genes. Further research is needed to better understand how Tas2r genes may influence diet selection in grazing ruminant species, which could provide more insight into management of western rangelands through sheep grazing strategies.

Keywords: sheep, Tas2r, annotation, comparative genomics

Introduction

Taste in grazing animals is perceived by taste receptors in cells found throughout the gustatory system (Chandrashekar et al., 2006). Of the five taste senses: bitter, sweet, sour, umami, and salty; bitterness is the most sensitive of the taste senses in sheep and cattle (Goatcher and Church, 1970). In plant toxicology, bitterness is often associated with toxins, and for grazing animals, the ability to detect these toxins is likely a mechanism used to prevent consumption of toxic plants and shrubs (Wong et al., 1996; Garcia-Bailo et al., 2009). The type two taste receptors (Tas2r) are G-protein-coupled with seven transmembrane regions (Striem et al., 1989) and are the only known receptors that can sense bitterness. In humans, Tas2r genes are suggested to be orthologous, typically 1000 base pairs, 300 – 330 amino acids, and intronless (Shi et al., 2003; Wang et al., 2004). It has been hypothesized that through evolution, species have lost function of some Tas2r genes and/or refined active genes (Lossow et al., 2016). Research on how taste receptors influence diet preferences has been limited to humans and mice to-date.

Diet preferences in grazing animals are known to be driven by several factors, including learned behavior (Provenza et al., 1992), environment (Launchbaugh et al., 1993), and genetic make-up (Garcia-Bailo et al., 2009), but little is known about how genetic variations within taste receptors effect diet preferences. Previous studies in humans have suggested that genetic variants in Tas2r can be linked to health issues, substance dependence, and other diseases (Drewnowski and Rock, 1995; Duffy and Bartoshuk, 2000).

One of the most well-known, and arguably the most studied, genetic variants within Tas2r genes is *TAS2R38*. Type two taste receptor 38 has several known haplotypes that result from three polymorphisms that substitute amino acids on position 49 (alanine to proline), 262 (valine to alanine), and 296 (isoleucine to valine) (Kim et al., 2003). Haplotype frequencies vary among ethnic populations, but two haplotypes that are predominately expressed categorize humans as tasters (PAV), and non-tasters (AVI) of 6-n-propyl-2-thiouracil (PROP), phenylthiocarbamide (PTC), or

other chemicals that contain thiourea moiety (Sandell and Breslin, 2006). Kim et al. (2003) determined that the haplotypes AVI and PAV were expressed within 96% of Europeans and 100% of East Asians within the studied population. Interestingly, some rare haplotypes were only identified in certain ethnic populations, such as PVI and AAI, which were found only in individuals with sub-Saharan Africa ancestry (Kim et al., 2003). The chemicals PROP and PTC elicit a bitter taste that differs in intensity depending on which haplotype is expressed by an individual. These haplotypes have also been associated with an individual's dietary preferences, particularly brassica vegetables, which also contain thiourea moiety (Sandell and Breslin, 2006). It has been theorized that individuals who perceive foods with intense bitterness will avoid them and substitute them with sweet foods (Keller and Tepper, 2004; Mennella et al., 2005). Subsequently, this may lead to weight gain and other health complications. Few studies have examined Tas2r genes in sheep, and there has been no research to-date investigating relationships between Tas2r genes and phenotypic traits, such as dietary preferences, in sheep. However, it is likely that sheep will exhibit similar genetic and phenotypic variation among individuals to those which have been observed in humans.

It has been suggested that Tas2r gene repertoires are similar within species that exhibit similar dietary habits (Dong et al., 2009). It is thus theorized that Tas2r gene repertoires within a species have likely experienced a series of gene loss and gene expansion to develop a repertoire that improves animal survivability (Go et al., 2005; Go, 2006; Dong et al., 2009). The number of functional Tas2r genes varies among species, which is also likely due to adaptation of consuming plants that contain different chemical compounds that express bitter-tasting attributes (Glendinning, 1994; Bachmanov et al., 2014). Furthermore, the toxicity that may be associated with these bitter-tasting compounds likely affects species differently, which would allow certain Tas2r genes to become obsolete in one species, but a vital gene for survival in another species.

This paper examines the genetic make-up of Tas2r gene repertoires in sheep (*Ovis aries*), cattle (*Bos taurus*), goat (*Capra hircus*), human (*Homo sapien*), dog (*Canus lupis familiaris),* and

mice (*Mus musculus*). The human and mice genomes are the most well-annotated, and therefore, their Tas2r repertoires are thought to be complete (Behrens and Meyerhof, 2013). Several studies have suggested that Tas2r gene expression can help predict dietary preferences of an individual and/or metabolic disease associated with diet (Tepper and Ullrich, 2002; Sandell and Breslin, 2006; Hayes et al., 2010). It is unknown whether the relationships in humans between Tas2r genes and diet preferences also exist in grazing livestock. Before extensive Tas2r genetic studies can be undertaken on grazing animals, particularly animals that graze vast rangelands, these genes must first be annotated.

Methods and Materials

Reference Genomes

Reference genomes used for comparison of Tas2r gene repertoires included *Ovis aries* (Oar_Rambouillet_v1.0), *Bos taurus* (ARS-UCD1.2), *Capra hircus* (ARS1), *Homo sapien* (GRCh38.p13), *Canis lupus familiaris* (CanFam3.1), and *Mus musculus* (GRCm38.p6).

Tas2r Gene Comparison

For Tas2r gene comparison of repertoires, reference genomes were examined through the National Center for Biotechnology Institute's "genome data viewer" (https://www.ncbi.nlm.nih.gov/genome/gdv/). Using "genome data viewer", each known and putative Tas2r gene for sheep, goat, cattle, human, mice, and dog were queried and the chromosome locations, sequences, number of base pairs, number of amino acids, presence of introns, and coding direction were recoded. No pseudo genes were recorded for comparison.

Similarity Comparison

Cluster 1 and 2 are the synteny block clusters found within a respective genome. Cluster 1 typically begins with *TAS2R16* and cluster 2 typically begins with *TAS2R42*. Using the NCBI gene database, Tas2r genes were located and FASTA (sequence of nucleotides or protein) files were downloaded to be used as input for gene comparison. Similarity comparisons of genes were conducted using FASTA files in "Multiple Sequence Alignment" *Muscle* by European Molecular

Biology Laboratory (EMBL-EBI) and analyzing the "percent identity summary" for similarity comparison. The phylogenetic tree was also created using *Muscle* results from FASTA files downloaded for each gene within the respective Tas2r repertoire. The phylogenetic tree and percent similarity matrix were constructed using all genes found within Cluster 2 of sheep, cattle, and goat, except for *TAS2R9, TAS2R8,* and *TAS2R7* since these genes were previously annotated for sheep, cattle, and goat. Additionally, genes of interest were blasted using NCBI's "nucleotide blast" *blastn* and "protein blast" *blastp* against the other species of interest. The closest hit of a comparative species was recorded with a threshold of e-value < 1e-10.

Protein Alignment

Protein sequences of genes of interest were aligned using "Cobalt", an alignment tool by NCBI. Within color selection option provided in Cobalt, "conservation" was chosen to demonstrate the conserved and non-conserved regions within genes of interest for compared species.

Results and Discussion

Dietary Variation Among Species

Dong (2009) suggested that animal species that share similar dietary habitats also share similar Tas2r repertoires. Sheep, cattle, and goats are all herbivores who dwell in the same ecological regions, however, they differ in diet selection dependent upon season (Coppock et al., 1986). Cattle are generalist grazers and primarily graze grasses (Schwartz and Ellis, 1981). Goats tend to graze selectively on forbs and browse on shrubs (Papachristou et al., 2005). Sheep are intermediate grazers and tend to graze on a mix of grasses, forbs, and shrubs (Schwartz and Ellis, 1981; Papachristou et al., 2005). While their general preferred dietary habits differ, there is often a high degree of overlap (Coppock et al., 1986). Humans, mice, and dogs are omnivores with vastly differing diets. The common house mouse tends to consume feeds by availability, dogs typically consume processed foods, and human diets vary first by availability and then by personal preferences for types of fats, protein, and sugars. While the two dominant haplotypes found within *TAS2R38* make up the majority of the human population, the rare *TAS2R38* haplotypes have primarily been found within sub-Saharan

African populations (Kim et al., 2003; Wooding et al., 2004). If the theory by Dong et al. (2009) is correct, it would suggest the difference in *TAS2R38* haplotypes is likely driven by limited variation of food availability within the African ecosystem that resulted in an adaptation of *TAS2R38* into the rare haplotype forms.

There is high similarity within the numerous Tas2r gene orthologs (Conte et al., 2002; Shi et al., 2003), and differences among Tas2r genes across species is likely driven by feeding ecology (Bachmanov et al., 2014). Differences in diet imposes an emphasis on certain genes that can detect toxins and improve survivability. Genes that are used to detect toxins within a diet are passed on to offspring, while genes that detect chemicals not found in a species' diet become unimportant for survival and are often lost through evolution (Dong et al., 2009). It has been suggested that some Tas2r genes have been evolutionarily tuned to broaden detection of several chemicals/toxins, while other Tas2r genes may have been finely tuned to detect only one chemical/toxin (Behrens and Meyerhof, 2013). For example, one Tas2r gene that may be of importance within grazing animals is *TAS2R16,* recognizes α-glucopyranosides, such as salicin (salicylic acid), which is found in willow bark and other shrubs (Bufe et al., 2002). Variations of *TAS2R16* may help predict grazing livestock avoidance of plants that contain salicylic acid (Raskin, 1992; Bufe et al., 2002) which has an unknown effect on grazing animals. However, the blood thinning qualities of salicylic acid (Link et al., 1943) would likely influence biological pathways.

Tas2r repertoire comparison

Of the species compared, Tas2r typically clustered in two regions, synteny blocks, with the exception of mice (Table 3.1). Mice Tas2r genes do not for synteny blocks like observed in the other species being compared and are mainly found on chromosome 6. This observation may be simply due to the fact mice have fewer chromosomes than the other species (mice – 20, human – 23, dog – 39, sheep – 27, goat – 30 and cattle – 30), and instead of having two synteny blocks on separate chromosomes, they are both found on chromosome 6. The genes found within mice chromosome 6

are ordered differently and several of the genes were not found within the other species compared (Table 3.1). Of the six species analyzed in this study, sheep, cattle, and goats have the most similar Tas2r repertoires (Table 3.1). Although, several genes were non-annotated within sheep, cattle, and goats, there were generally a total of 20 - 23 Tas2r genes within both clusters, and typically contained the same genes. Furthermore, the genes had a similar number of nucleotides and amino acids (Table 3.2).

Tas2r Cluster 1

Cluster 1 was located on the same chromosome number (4) for sheep, cattle, and goats, but different chromosomes for human (7), dog (16), and mice (6). Within Cluster 1, sheep, cattle, goats, humans, and dogs exhibited the same genes, except for *TAS2R134-like,* which was absent in humans and *TAS2R16* was absent in dogs. The same genes within Cluster 1 were found in sheep, cattle, goat, human, and dog, but the gene order was reversed in goat and dog. It is not known if the difference in the order of the genes has an effect on gene expression (Table 3.1).

Interestingly, within Cluster 1, sheep *TAS2R5* and dog *TAS2R60* had introns according to NCBI's genome data viewer, which was different from previous research in humans where Tas2r genes were described as intronless (Shi et al., 2003; Wang et al., 2004). It is unknown whether Tas2r genes containing introns correlates with a difference in phenotypic traits. Within Cluster 1, Tas2r genes tended to have a length of 900-1100bp and 300-330 amino acids (Table 3.2). The only exceptions were sheep *TAS2R*3, *TAS2R*4, and *TAS2R*5, which according to the reference sequence information, were made up of 2841, 1710, and 2645 bp, respectively. The increased number of base pairs in *TAS2R*3 and *TAS2R*4 was due to untranslated regions and the increased number of base pairs in *TAS2R5* was due to the presence of introns.

Tas2r gene similarity comparison Cluster 1

The similarity comparisons (Table 3.3) between the species of Tas2r genes found within Cluster 1 indicated that sheep and goat Tas2r genes were most similar (> 97%), followed by cattle

compared to sheep and goats (both > 90%), and human compared to dog (> 76%). Mice were dissimilar to all other species (< 71%). These observations support the theory proposed by Dong et al. (2009) that species with similar dietary preferences also have similar Tas2r genes. It is possible, based on their homology, that sheep, goats, and cattle are adequate models for comparisons among each other, and that a relationship observed in one species may also be observed in a species with similar homology.

While absent in humans, there was only one non-annotated gene within Cluster 1 that was shared across the other compared species. This gene was labeled as "134-like" or "143-like" (Table 3.2), but due to high similarity (97.61%), it is likely the same gene in sheep and goats (Table 3.3). This is not the only case of incomplete annotation of Tas2r genes for sheep and goats. Unlike Cluster 1 where the majority of genes have been annotated, in Cluster 2 of sheep and goat the majority of genes were non-annotated.

Tas2r Cluster 2

Different from Cluster 1, Cluster 2 was found on chromosome 3 for sheep, chromosome 5 for cattle and goat, chromosome 12 for human, and chromosome 27 for dog (Table 3.1). However, it was notable that Cluster 2 was found within metacentric chromosomes in sheep and not cattle or goat. Although the Tas2r genes in Cluster 2 have not been completely annotated in sheep and goat, they were similar among sheep, cattle, goat, and dog. Furthermore, Cluster 2 on human chromosome 12 contained the same genes as the other species, but with 10 additional genes and the gene order was reversed compared with sheep, cattle, goat, and dog (Table 3.1).

The Tas2r genes within Cluster 2 were of similar length to those found in Cluster 1, with the exception of cattle LOC100140395, which contains 3159 base pairs (Table 3.4). The increased number of nucleotides may be due to untranslated intron regions. The *TAS2R9* gene within Cluster 2 also had introns in sheep and cattle (Table 3.4).

Tas2r gene similarity comparison Cluster 2

Similarity comparisons of genes in Cluster 2 yielded similar results to those of Cluster 1 (Table 3.5). Sheep and goat had the greatest similarity (> 95%), followed by cattle compared to sheep and goats (> 94%), human compared to dogs (> 78%), and mice were dissimilar to the rest of the species (< 59%).

Ferreira et al. (2012) first discovered eight sheep Tas2r genes through the use of comparative genomics and by blasting known Tas2r cattle sequences to the sheep genomes. Moreover, their findings suggested that sheep and cattle Tas2r genes had a similarity of > 92%. Similarly, in this study, all twenty Tas2r genes in sheep had a similarity of > 91.9% when compared with cattle. Not surprisingly, sheep and goats exhibited an even higher similarity of > 95.5%. The dissimilarity of mice compared with the other species is likely due to the evolution of Tas2r genes (Dong et al., 2009).

Proposed annotations of sheep, cattle, and goat in Cluster 1

TAS2R62. Within Cluster 1, there was one gene that was non-annotated and likely shared among sheep LOC101102056 (*TAS2R134-like*), cattle LOC785618 (*TAS2R134-like*), and goat LOC102185432 (*TAS2R143-like*). When the sheep LOC101102056 (*TAS2R134-like*) nucleotide sequence was blasted against human, dog, and mice, the results did not meet blast criteria. Although, the blast results within NCBI's database among all of the species compared would indicate that the *TAS2R134-like* genes should be annotated as *TAS2R62* for sheep, cattle, and goat (Appendix Figure 1-3).

TAS2R41. This gene was already annotated within sheep and cattle, but the order of genes within synteny block Cluster 1 would indicate that goat LOC102185981 should actually be *TAS2R41*. This observation was supported by blast results (Table 3.6) and percent similarity (Table 3.3). Therefore, the proposed annotation of goat LOC102185981 is *TAS2R41*.

Proposed annotations of sheep, cattle, and goat in Cluster 2

Within Cluster 2, there were six non-annotated genes in sheep, two in cattle, and nine in goat. Only three genes within Cluster 2 were annotated the same for sheep, cattle, and goat *(TAS2R7, TAS2R8,* and *TAS2R9).* For the purpose of annotating the seventeen non-annotated genes, each gene within Cluster 2 for sheep, cattle, and goat were nucleotide and protein blasted (unless the gene was absent in sheep, or already annotated; Table 3.6), compared for percent similarity (Table 3.7), and observed in a phylogenetic tree (Figure 3.1). The proposed annotations for each non-annotated gene are depicted in Table 8 and are discussed below.

TAS2R42. The first gene (or last gene, depending upon the order of Tas2r genes for each species) within Cluster 2 was *TAS2R42*. A *TAS2R42* gene was already annotated in sheep and cattle; however, when blasted, observed in a phylogenetic tree, and compared for percent similarity, these genes were not the best possible match to *TAS2R42*. Cattle had a non-annotated gene LOC100140395 with the nucleotide name "*TAS2R42-Like*" that was 97.21% similar to sheep *TAS2R42,* 96.89% similar to goat Loc102169081, and was located before cattle *TAS2R42*. Cattle LOC100140395 blasted to sheep *TAS2R42* (97.21%), goat LOC102169081 (96.89%), human *TAS2R42* (73.51%), dog *TAS2R42* (79.45%), and mice *Tas2r31* (64.45%; mice do not have *Tas2r42*). The low query scores for blast results from proposed *TAS2R42* can be attributed to the size of cattle LOC100140395, which was 3-fold larger than *TAS2R42* in the other species. Cattle Tas2r gene LOC100140395 also best matched with sheep *TAS2R42* and Goat LOC102169081 in the phylogenetic tree and percent similarity matrix (> 97.31%). Therefore, the proposed annotation is to remove the annotation of cattle *TAS2R42* and annotate cattle LOC100140395 and goat LOC102169081 as *TAS2R42.* No change is proposed for sheep *TAS2R42*.

TAS2R67. Sheep LOC101120486 blasted to cattle *TAS2R42* (95.74%; prior to proposed annotation of cattle LOC100140395 as *TAS2R42* described above), goat LOC102169365 (98.30%), human *TAS2R67* pseudo gene (76.50%), dog *TAS2R67* (79.34%), and no Tas2r genes in mice met the

blast criteria. Therefore, the proposed annotation is to annotate sheep LOC101120486 and goat LOC102169365 as *TAS2R67,* as well as to change the annotation of cattle *TAS2R42* to *TAS2R67*.

TAS2R67B. Sheep LOC101120742 blasted to cattle *TAS2R42* (93.08%; prior to proposed annotation of cattle LOC100140395 as *TAS2R42* described above), goat LOC102169365 (95.19%), human *TAS2R67pseudo* (76.30%), dog *TAS2R67* (77.91%), and no mice Tas2r genes met the blast criteria. Although sheep LOC101120742 and sheep *TAS2R67* both blasted to the same genes in cattle, human, and dog, all blast results were lower in percent identity and total score for sheep LOC101120742 compared to the results of sheep *TAS2R67*. Grouping observed in the phylogenetic tree and the percent similarity comparison indicated that sheep LOC101120742 and goat LOC102169365 are likely genes that are not possessed by cattle, human, dog, and mice. Therefore, the proposed annotation is to annotate sheep LOC101120742 and goat LOC102169365 as *TAS2R67B*.

TAS2R46. Goat LOC1021179923 blasted to cattle *TAS2R46* (94.66%), sheep *Tas2r46 pseudo* (88.20%), human *TAS2R50* (72.74%), and dog *TAS2R43* (76.19%), and no mice Tas2r genes met the blast criteria. The goat LOC1021179923 blasted best to human *TAS2R50* with a similarity of 72.74% and to human *TAS2R46* with a similarity of 71.78%. Dog also did not have a *TAS2R46*. The grouping observed in the phylogenetic tree and the percent similarity comparison indicated that goat LOC1021179923 is likely the same gene as cattle *TAS2R46*. Therefore, the proposed annotation is to annotate goat LOC1021179923 as *TAS2R46*.

TAS2R31. Sheep LOC101121003 blasted to cattle LOC782957(96.08%), goat LOC102169653 (97.82%), human *TAS2R30* (74.75%), and there were no Tas2r genes for dog and mice that met the blast criteria. Sheep LOC101121003, cattle LOC782957, and goat LOC102169653 all share the same nucleotide name of *"TAS2R31".* Although human *TAS2R30* blasted with the highest similarity of 74.93%, human *TAS2R31* had a similarity of 74.32%. Therefore, the proposed annotation is to annotate sheep LOC101121003, cattle LOC782957, and goat LOC102169653 as *TAS2R31*.

TAS2R43. Goat LOC102169944 blasted to a sheep *TAS2R43pseudo* (86.67%), cattle *TAS2R43pseudo* (94.28%), human *TAS2R30* (74.93%), dog *TAS2R43* (79.47%), and no Tas2r genes for mice met the blast criteria. Although goat LOC102169944 blasted to human *TAS2R30* with a similarity of 74.93%, human *TAS2R43* had a similarity of 74.43%. The phylogenetic tree agrees with the blast results, which suggested that sheep and cattle may no longer have *TAS2R43*. Therefore, the proposed annotation is to annotate goat LOC102169944 as *TAS2R43*.

TAS2R12. Sheep LOC10114857 blasted to cattle *TAS2R12* (95.09%), goat LOC102180198 (99.23%), human *TAS2R12pseudo* (69.45%), dog *TAS2R12* (76.67%), and mice *tas2r22* (65.69%; mice do not have *TAS2R12)*. The name associated with sheep LOC10114857 and goat LOC102180198 was *"TAS2R7"*; however, sheep and goat already had an annotated *TAS2R7* and blast results indicated the best match was *TAS2R12*. Therefore, the proposed annotation is to annotate sheep LOC10114857 and goat LOC102180198 as *TAS2R12.*

TAS2R10B. Sheep LOC101115110 blasted to cattle *TAS2R10B* (94.86%), goat LOC102180468 (99.35%), human *TAS2R10* (77.73%), dog *TAS2R10* (99.53%), and mice *Tas2r5* (69.21%). Therefore, the proposed annotation is to annotate sheep LOC101115110 and goat LOC102180198 as *TAS2R10B*.

TAS2R10. Sheep LOC101122269 blasted to cattle *TAS2R10* (95.33%), goat LOC102181009 (98.56%), human *TAS2R10* (78.05%), dog *TAS2R10* (80.44%), and mice *Tas2r5* (70.15%). Therefore, the proposed annotation is to annotate sheep LOC101122269 and goat LOC102181009 as *TAS2R10*.

The last two proposed annotations, sheep and goat *TAS2R10* and *TAS2R10B*, both blasted to *TAS2R10* in human and dog. Cattle were already annotated for *TAS2R10* and *TAS2R10B*. The proposed annotations for sheep and goat *TAS2R10* and *TAS2R10B* had the greatest similarity to cattle *TAS2R10* (≥95.33%) and *TAS2R10B* (≥94.95%), respectively.

Protein alignment

Two genes that may be of particular interest in grazing animals are *TAS2R38* and *TAS2R16*. The alignment of *TAS2R38* protein sequences within the six species observed suggested that *TAS2R38* is highly conserved (Figure 3.2). Amino acid positions 49, 262, and 296 are of particular interest as these amino acid substitutions predict haplotypes found within humans (Kim et al., 2003). Although highly conserved, there were some differences of amino acids in the positions that dictate haplotypes in humans, compared to the reference genomes of other species. The amino acid at position 49 in humans was alanine, but for all of the other species compared, proline was in position 49. The amino acid in position 262 was alanine for all of the species except dog, which expressed valine at position 262. The amino acid in position 296 was isoleucine for all of the species compared. Interesting, within the grazing animals, sheep, cattle, and goat, each of the amino acids found in positions 49, 262, and 296 were the same. The protein alignment of *TAS2R38* also suggested insertions or deletions at positions 177-179 (Figure 3.3). As suggested by Kim et al. (2003), haplotypes may be affecting the Tas2r G-protein orientation, which would limit the ability for ligands to bind to their receptors. The insertions and deletions observed at positions 177-179 in *TAS2R38* may also play an important role in functionality of the gene and/or orientation of the G-proteins. Further research is needed to determine if *TAS2R38* exhibits similar phenotypic relationships, such as prediction of diet preferences, in grazing animals to those observed in humans.

A similar observation of high conservation was present within the protein alignment of *TAS2R16* (Figure 3.4). Dog was excluded from *TAS2R16* alignment due to the absence of the gene. Although highly conserved, the protein alignment suggested a deletion in humans at position 182 and position 205 that was not expressed in the *TAS2R16* protein sequences for sheep, cattle, goat, and mice (Figure 3.5). Mice also had a deletion at position 213 (Figure 3.5). Similar to *TAS2R38,* further research is needed to determine if these sequence variations among species affect the gene function of *TAS2R16*.

Conclusion

The adaptation of Tas2r genes to influence a species' dietary selection is likely very complex. Variations within Tas2r genes have been recorded in humans and mice, but there has been little research to-date investigating genetic variation of Tas2r genes in grazing animals. In humans, bitter taste categories have been assigned to individuals as tasters, non-tasters and super tasters depending on their taste response to phenylthiocarbamide (PTC), which can be predicted by an individual's haplotype on *TAS2R38* (Bartoshuk et al., 1994; Kim et al., 2003). Henslee et al. (2019) observed a similar response while testing mature Rambouillet and Targhee rams for avoidance of PTC. Avoidance of PTC was highly variable between individual rams, and individual animals could be categorized into three PTC consumption groups including high, intermediate, and low, which are similar to the findings in humans' taste avoidance of PTC. These data suggested that there may also be genetic differences within *TAS2R38* or other Tas2r genes in sheep that influence avoidance of feeds that contain thiourea moiety. Extensive research is required within sheep to determine if the variants in the Tas2r genes are correlated with diet preferences. The completed annotation of Tas2r gene repertoires may be the first step in determining genetic influences of diet preferences. Genetic predictions of dietary preferences could provide insights into livestock grazing behavior on rangelands with varying plant communities and improved understanding of targeted grazing practices.

Tas2r genes have been extensively studied in humans, and have been genetically correlated with dietary preferences (Drewnowski and Rock, 1995), body mass index (Tepper and Ullrich, 2002), substance dependence (Hinrichs et al., 2006), thyroid disease (Clark et al., 2014), and most recently, upper respiratory immunology (Douglas and Cohen, 2017). In addition to the influences that Tas2r genes may have on diet selection in grazing animals, understanding how Tas2r genes may affect immune function and/or disease susceptibility would lead to improved production management strategies and overall animal health.

Implications

Understanding the role Tas2r genes have on traits and diseases in humans has become of significant importance. In animal production, Tas2r genes could be just as important. Identification of Tas2r genes may not only be used to predict dietary preferences of individual animals, but also provide a better understanding of the ways in which individual animals can utilize low-quality forages when they are not averse to the taste. Low-quality forages can be found globally, and selection for individuals whose dietary preferences are not to avoid these forages could improve animal production, especially in third world countries.

The completed annotation of the sheep, cattle, and goat Tas2r repertoires will allow for further research into how Tas2r genes may influence dietary selection in grazing animals. Moreover, it may allow for selection of animals based on their genotype for grazing strategies to improve rangelands. Similar genetic structures of Tas2r genes between grazing animals may result in similar phenotypic relationships, which would suggest that new information related to Tas2r repertoires in sheep would likely also be observed in goat and cattle.

Tables and Figures

Table 3.1. Type two taste receptor gene repertoires by specie, ordered from top to bottom in sequence within their respective cluster (Cl.) and chromosome (Ch.).

Specie	Sheep		Cattle		Goat		Human			Dog		Mice		
Cl.	**1**	**2**	**1**	**2**	**1**	**2**		**1**	**2**	**1**	**2**			
Ch.	**4**	**3**	**4**	**5**	**4**	**5**	**5**	**7**	**12**	**16**	**27**	**2**	**6**	**15**
	16	42	16	*Loc100140395*	*Loc102185981*	*Loc102169081*	1	16	7	41	42	34	18	19
	3	*Loc101120486*	3	42	60	*Loc102179076*		3	8	60	67		37	
	4	*Loc101120742*	4	46	*Loc102185432*	*Loc102169365*		4	9	*Loc608741*	43		8	
	5	*Loc101121003*	5	*Loc782597*	40	*Loc102179923*		5	10	40	*Loc100682759*		38	
	38	*Loc101114857*	38	65	39	*Loc102169653*		38	12	39	12		43	
	39	*Loc101115110*	39	12	38	*Loc102169944*		39	13	38	10		35	
	40	*Loc101122269*	40	10B	5	*Loc102180198*		40	14	5	9		26	
	Loc101102056	9	*Loc785618*	10	4	*Loc102180468*		60	15	4	8		30	
	60	8	60	10C	3	*Loc102181009*		41	50	3	7		7	
	41	7	41	9	16	9			20				6	
				8		8			19				4	
				7		7			31				5	
									63				14	
									46				20	
									64				21	
									43				22	
									30				15	
									18				24	
									42				2	
													36	
													17	
													23	
													16	
													10	
													13	
													25	
													29	
													31	
													9	
													3	
													40	

Table 3.2. Comparison of type two taste receptor gene (Tas2r) genes found in Cluster 1 of sheep with Tas2r genes in cattle, human, goat, dog and mice. Chromosome location indicated by "Ch", number of base pairs "Bp", number of amino acids "AA", if gene is intronless "IL" ("Y" = yes; "N" = no), and direction the gene is coded "CD" ("F" = forward' "R" = reverse).

Tas2r	**16**					**3**					**4**					**5**					**38**				
	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD
Sheep	4	928	301	Y	R	4	2841	316	Y	F	4	1710	296	Y	F	4	2645	311	N	F	4	1002	333	Y	R
Cattle	4	906	301	Y	R	4	951	316	Y	F	4	891	296	Y	F	4	878	293	Y	F	4	1045	335	Y	R
Goat	4	944	311	Y	F	4	1583	316	Y	R	4	891	296	Y	R	4	875	291	Y	R	4	1008	335	Y	F
Human	7	996	291	Y	R	7	1101	316	Y	F	7	900	299	Y	F	7	1150	299	Y	F	7	1143	333	Y	R
Mice	6	918	305	Y	F	6	939	312	Y	R	6	909	302	Y	R	6	903	300	Y	R	6	996	331	Y	R
Dog	-	-	-	-	-	16	951	316	Y	R	16	1026	299	Y	R	16	888	295	Y	R	16	951	316	Y	F
Tas2r	**39**					**40**					**134 and 143* Like**					**60**					**41**				
	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD
Sheep	4	1092	353	y	F	4	1364	318	Y	F	4	881	293	Y	F	4	954	317	Y	F	4	936	311	Y	F
Cattle	4	1146	381	Y	F	4	2635	318	Y	F	4	926	308	Y	F	4	954	317	Y	F	4	894	297	Y	F
Goat	4	1149	382	Y	R	4	1023	317	Y	R	4*	924	307	Y	R	4	954	317	Y	R	4	936	311	Y	R
Human	7	1017	338	Y	F	7	1043	323	Y	F	-	-	-	-	-	7	957	318	Y	F	7	924	307	Y	F
Mice	6	960	319	Y	F	6	939	312	Y	R	6*	882	293	Y	F	-	-	-	-	-	-	-	-	-	-
Dog	16	963	320	Y	R	16	921	306	Y	R	16*	1044	301	Y	R	16	821	273	N	F	16	927	308	Y	R

Table 3.3. Nucleotide sequence percent similarity comparison of Tas2r genes found on sheep Cluster 1 compared to goat, human, cattle, dog and mice.

			TAS2R16			
Mice16	100.00	49.88	47.65	47.89	47.66	
Human16	49.88	100.00	74.22	73.52	73.12	
Cattle16	47.65	74.22	100.00	94.48	94.37	
Sheep16	47.89	73.52	94.48	100.00	99.46	
Goat16	47.66	73.12	94.37	99.46	100.00	
			TAS2R3			
Mice3	100.00	57.01	60.10	59.38	58.82	58.94
Human3	57.01	100.00	80.44	79.18	77.66	77.48
Dog3	60.10	80.44	100.00	81.28	81.70	81.49
Cattle3	59.38	79.18	81.28	100.00	96.64	96.42
Sheep3	58.82	77.66	81.70	96.64	100.00	98.73
Goat3	58.94	77.48	81.49	96.42	98.73	100.00
			TAS2R4			
Mice4	100.00	56.61	56.84	56.43	53.35	52.25
Sheep4	56.61	100.00	99.10	97.31	80.70	81.65
Goat4	56.84	99.10	100.00	97.31	80.92	82.21
Cattle4	56.43	97.31	97.31	100.00	81.93	82.21
Human4	53.35	80.70	80.92	81.93	100.00	82.27
Dog4	52.25	81.65	82.21	82.21	82.27	100.00
			TAS2R5			
Mice5	100.00	48.82	48.82	49.35	47.66	48.77
Cattle5	48.82	100.00	94.95	94.95	79.16	81.14
Sheep5	48.82	94.95	100.00	97.81	79.14	82.31
Goat5	49.35	94.95	97.81	100.00	79.77	82.22
Human5	47.66	79.16	79.14	79.77	100.00	85.36
Dog5	48.77	81.14	82.31	82.22	85.36	100.00
			TAS2R38			
Mice38	100.00	74.50	73.64	74.00	76.51	76.22
Cattle38	74.50	100.00	95.61	95.54	76.27	79.81
Sheep38	73.64	95.61	100.00	98.70	76.45	79.50
Goat38	74.00	95.54	98.70	100.00	76.79	79.50
Human38	76.51	76.27	76.45	76.79	100.00	79.81
Dog38	76.22	79.81	79.50	79.50	79.81	100.00
			TAS2R39			

Mice39	100.00	67.89	67.05	67.26	71.77	70.84
Cattle39	67.89	100.00	96.79	96.42	78.37	83.07
Sheep39	67.05	96.79	100.00	99.27	78.51	83.07
Goat39	67.26	96.42	99.27	100.00	78.47	83.07
Human39	71.77	78.37	78.51	78.47	100.00	83.54
Dog39	70.84	83.07	83.07	83.07	83.54	100.00
			TAS2R40			
Mice40	100.00	56.61	57.34	55.34	54.70	53.82
Cattle40	56.61	100.00	95.81	95.69	81.21	83.13
Sheep40	57.34	95.81	100.00	97.55	80.74	82.07
Goat40	55.34	95.69	97.55	100.00	80.72	82.46
Human40	54.70	81.21	80.74	80.72	100.00	84.47
Dog40	53.82	83.13	82.07	82.46	84.47	100.00
			TAS2R143-like			
Mice143-like	100.00	71.32	69.26	69.03	69.53	
Dog143-like	71.32	100.00	77.54	76.84	77.06	
Cattle134-like	69.26	77.54	100.00	91.90	92.28	
Sheep134-like	69.03	76.84	91.90	100.00	97.61	
Goat143-like	69.53	77.06	92.28	97.61	100.00	
			TAS2R60			
Dog60	100.00	74.92	73.33	73.23	73.01	
Human60	74.92	100.00	75.89	75.47	76.00	
Cattle60	73.33	75.89	100.00	94.55	94.34	
Sheep60	73.23	75.47	94.55	100.00	98.74	
Goat60	73.01	76.00	94.34	98.74	100.00	
			TAS2R41			
Human41	100.00	76.30	76.32	76.41	75.65	
Dog41	76.30	100.00	79.08	79.07	78.86	
Cattle41	76.32	79.08	100.00	94.85	94.41	
Goat41	76.41	79.07	94.85	100.00	98.72	
Sheep41	75.65	78.86	94.41	98.72	100.00	

Table 3.4. Comparison of type two taste receptor gene (Tas2r) genes found on Cluster 2 of sheep with Tas2r genes in cattle, human, goat, dog and mice. Chromosome location is indicated by "Ch", number of base pairs "Bp", number of amino acids "AA", if gene is intron less "IL" ("Y" for yes and "N" for no), and the direction the gene is coded "CD" ("F" for forward and "R" for reverse).

Tas2r	**42**					**67**					**67B**					**31**					**12**				
	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD
Sheep	3	930	309	Y	F	3	939	312	Y	F	3	936	311	Y	F	3	914	304	Y	F	3	912	303	Y	F
Cattle	5	3159	309	Y	F	5	932	312	Y	F	-	-	-	-	-	5	888	295	Y	F	5	930	309	Y	F
Goat	5	931	309	Y	F	5	939	312	Y	F	5	1039	290	Y	F	5	918	305	Y	F	5	912	303	Y	F
Human	12	945	314	Y	F	-	-	-	-	-	-	-	-	-	-	12	1021	309	Y	R	12	948	-	Y	R
Mice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	933	310	Y	R	-	-	-	-	-
Dog	27	972	323	Y	F	27	932	312	Y	F	-	-	-	-	-	-	-	-	-	-	27	945	314	Y	F
Tas2r	**10B**					**10A**					**9**					**8**					**7**				
	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD	Ch	Bp	AA	IL	CD
Sheep	3	930	309	Y	F	3	900	299	Y	F	3	1164	327	N	Y	3	929	309	Y	F	3	939	312	Y	F
Cattle	5	933	310	Y	F	5	900	299	Y	F	5	1272	363	N	F	5	930	309	Y	F	5	939	312	Y	F
Goat	5	930	309	Y	F	5	900	299	Y	F	5	935	311	Y	F	5	995	331	Y	F	5	939	312	Y	F
Human	-	-	-	-	-	12	924	307	Y	R	12	1075	312	Y	R	12	930	309	Y	R	12	1096	318	Y	R
Mice	-	-	-	-	-	6	1002	333	Y	F	6	951	316	Y	R	6	894	297	Y	F	6	1032	308	Y	R
Dog	-	-	-	-	-	27	969	322	Y	F	27	843	280	Y	F	27	919	305	Y	F	27	939	312	Y	F

Table 3.5. Nucleotide sequence percent similarity comparison of T2R genes found on Cluster 2 of sheep compared to goat, human, cattle, dog and mice.

			TAS2R42			
Human42	100.00	77.78	74.19	74.30	74.30	
Dog42	77.78	100.00	80.86	80.54	80.65	
Cattle0395 (42)	74.19	80.86	100.00	96.99	97.31	
Goat9081 (42)	74.30	80.54	96.99	100.00	99.25	
Sheep42	74.30	80.65	97.31	99.25	100.00	
			TAS2R67			
Cattle42 (67)	100.00	95.95	95.63			
Sheep0486 (67)	95.95	100.00	98.30			
Goat9076 (67)	96.63	98.30	100.00			
			TAS2R67B			
Goat9365 (67B)	100.00	95.52				
Sheep0742 (67B)	95.52	100.00				
			TAS2R31			
Mice31	100.00	55.63	57.19	56.84	56.72	
Human31	55.63	100.00	74.32	74.20	73.88	
Cattle2597 (31)	57.19	74.32	100.00	96.50	96.30	
Sheep1003 (31)	56.84	74.20	96.50	100.00	98.25	
Goat9653 (31)	56.72	73.88	96.30	98.25	100.00	
			TAS2R12			
Dog12	100.00	78.37	77.74	77.63		
Cattle12	78.37	100.00	95.29	95.39		
Goat0198	77.74	95.29	100.00	99.23		
Sheep4857	77.63	95.39	99.23	100.00		
			TAS2R10B			
Cattle10B	100.00	95.05	95.70			
Sheep5110 (10B)	95.05	100.00	99.35			
Goat0468 (10B)	95.70	99.35	100.00			
			TAS2R10			
Mice10	100.00	56.58	56.80	56.80	56.62	56.53
Cattle10	56.58	100.00	95.67	95.33	78.08	81.11
Goat1009 (10)	56.80	95.67	100.00	98.56	77.74	80.78
Sheep2269 (10)	56.80	95.33	98.56	100.00	77.74	80.56
Human10	56.62	78.08	77.74	77.74	100.00	83.97
Dog10	56.53	81.11	80.78	80.56	83.97	100.00
			TAS2R9			

Mice9	100.00	55.05	55.56	55.94	57.43	56.88
Cattle9	55.05	100.000	94.59	94.55	77.46	78.50
Sheep9	55.56	94.59	100.00	97.75	77.67	79.26
Goat9	55.94	94.55	97.75	100.00	78.29	79.08
Human9	57.43	77.46	77.67	78.29	100.00	83.04
Dog9	56.88	78.50	79.26	79.08	83.04	100.00
			TAS2R8			
Mice8	100.00	49.94	51.49	51.58	51.88	51.64
Human8	49.94	100.00	78.00	76.13	75.35	75.67
Dog8	51.49	78.00	100.00	80.28	79.93	80.48
Cattle8	51.58	76.13	80.28	100.00	94.19	94.40
Sheep8	51.88	75.35	79.93	94.19	100.00	97.20
Goat8	51.64	75.67	80.48	94.40	97.20	100.00
			TAS2R7			
Mice7	100.00	57.98	58.20	58.64	58.61	59.96
Cattle7	57.98	100.00	97.12	97.44	81.90	84.98
Sheep7	58.20	97.12	100.00	99.47	81.79	84.88
Goat7	58.64	97.44	99.47	100.00	81.47	84.77
Human7	58.61	81.90	81.79	81.47	100.00	85.30
Dog7	59.96	84.98	84.88	84.77	85.30	100.00

Table 3.6. Blast results for proposed annotation of Tas2r genes in sheep, cattle, and goat. Blasted specie is indicated by "*" and gene name proposed to be changed **bolded**.

		Nucleotide *Blastn*					Protein *Blastp*				
Proposed annotation	Blasted	Best alignment	Total score	QC	E-value	% Iden.	Best alignment	Total score	QC	E-value	% Iden.
TAS2R41	Goat*	***LOC102185981***									
	Sheep	*TAS2R41*	1661	100%	0.0	98.72%	*TAS2R41*	610	100%	0.0	97.75%
	Cattle	*TAS2R41*	1391	95%	0.0	94.75%	*TAS2R41*	543	98%	0.0	92.13%
	Human	-	-	-	-	-	*TAS2R41*	375	92%	2e-127	67.13%
	Dog	*TAS2R41*	688	95%	0.0	80.69%	*TAS2R41*	448	99%	3e-156	74.68%
	Mice	-	-	-	-	-	*TAS2R41*	413	99%	2e-142	67.53%
TAS2R42	Cattle*	***LOC100140395***					***LOC100140395***				
	Sheep	*TAS2R42*	1559	29%	0.0	97.21%	*TAS2R42*	558	100%	0.0	95.79%
	Goat	***LOC102169081***	1545	29%	0.0	96.89%	***LOC102169081***	559	100%	0.0	95.79%
	Human	*TAS2R42*	565	28%	2e-157	73.51%	*TAS2R42*	300	100%	7e-98	57.01%
	Dog	*TAS2R42*	847	30%	0.0	79.45%	*TAS2R42*	356	100%	8e-120	68.71%
	Mice	*tas2r31*	137	27%	3e-28	64.45%	-	-	-	-	-
TAS2R67	Sheep*	***LOC101120486***					***LOC101120486***				
	Cattle	***TAS2R42***	1514	100%	0.0	95.74%	***TAS2R42***	515	100%	0.0	91.67%
	Goat	***LOC102179076***	1622	100%	0.0	98.30%	***LOC102179076***	600	100%	0.0	96.47%
	Human	*TAS2R67psuedo*	700	99%	0.0	76.50%	-	-	-	-	-
	Dog	*TAS2R67*	820	100%	0.0	79.34%	*TAS2R67*	338	100%	7e-113	68.27%
	Mice	-	-	-	-	-	-	-	-	-	-
TAS2R67B	Sheep*	***LOC101120742***					***LOC101120742***				
	Cattle	*TAS2R42*	1399	100%	0.0	93.08%	*TAS2R42*	459	100%	1e-174	88.14%
	Goat	***LOC102169365***	1384	92%	0.0	95.19%	***LOC102169365***	469	92%	1e-164	91.72%

	Human	*TAS2R67psuedo*	686	99%	0.0	76.30%	-	-	-	-	-
	Dog	*Tas2r67*	800	100%	0.0	77.91%	*Tas2r67*	338	100%	1e-112	67.63%
	Mice	-	-	-	-	-	-	-	-	-	-
TAS2R46	Goat*	***LOC102179923***					***LOC102179923***				
	Cattle	*46*	1429	100%	0.0	94.66%	*46*	513	100%	0.0	89.51%
	Sheep	*TAS2R46pseudo*	1111	99%	0.0	88.20%	-	-	-	-	-
	Human	*TAS2R50*	516	99%	1e-142	72.74%	-	-	-	-	-
	Dog	*TAS2R43*	642	99%	0.0	76.19%	*TAS2R43*	336	99%	2e-111	58.09%
	Mice	-	-	-	-	-	-	-	-	-	-
TAS2R31	Sheep*	***LOC101121003***					***LOC101121003***				
	Cattle	***LOC782957***	1493	100%	0.0	96.08%	***LOC782957***	564	100%	0.0	92.13%
	Goat	***LOC102169653***	1565	100%	0.0	97.82%	***LOC102169653***	584	100%	0.0	96.07%
	Human	*TAS2R30*	590	95%	2e-165	74.75%	*TAS2R49*	334	98%	2e-111	58.36%
	Dog	-	-	-	-	-	*TAS2R43*	381	98%	3e-129	64.90%
	Mice	-	-	-	-	-	-	-	-	-	-
TAS2R43	Goat*	***LOC102169944***					***LOC102169944***				
	Cattle	*TAS2R43pesedo*	1184	98%	0.0	94.28%	***LOC782957***	357	100%	3e-121	71.43%
	Sheep	*TAS2R43pseudo*	895	96%	0.0	86.67%	***LOC102169653***	345	97%	9e-117	71.83%
	Human	*TAS2R30*	493	96%	3e-136	74.93%	-	-	-	-	-
	Dog	*TAS2R43*	652	96%	0.0	79.47%	*TAS2R43*	283	100%	1e-91	59.07%
	Mice	-	-	-	-	-	-	-	-	-	-
TAS2R12	Sheep*	***LOC101114857***					***LOC101114857***				
	Cattle	*TAS2R12*	1449	100%	0.0	95.09%	*TAS2R12*	514	98%	0.0	90.97%
	Goat	***LOC102180198***	1614	100%	0.0	99.23%	***LOC102180198***	590	100%	0.0	98.68%
	Human	*TAS2R12psuedo*	353	90%	7e-94	69.45%	*TAS2R7*	195	99%	1e-56	40.45%
	Dog	*TAS2R12*	686	97%	0.0	76.67%	*TAS2R12*	354	98%	3e-119	61.56%
	Mice	*tas2r22*	190	89%	5e-45	65.69%	*tas2r22*	244	96%	5e-76	48.68%

TAS2R10B	Sheep*	***LOC101115110***					***LOC101115110***				
	Cattle	*TAS2R10B*	1465	100%	0.0	94.86%	*TAS2R10B*	541	100%	0.0	88.71%
	Goat	***LOC102180468***	1651	100%	0.0	99.35%	***LOC102180468***	615	100%	0.0	99.35%
	Human	*TAS2R10*	712	99%	0.0	77.73%	*TAS2R10*	359	100%	3e-121	60.84%
	Dog	*TAS2R10*	1503	90%	0.0	99.53%	*TAS2R10*	410	98%	3e-141	67.21%
	Mice	*tas2r5*	342	96%	1e-90	69.21%	-	-	-	-	-
TAS2R10	Sheep*	***LOC101122269***					***LOC101122269***				
	Cattle	*TAS2R10*	1434	100%	0.0	95.33%	*TAS2R10*	556	100%	0.0	91.97%
	Goat	***LOC102181009***	1565	100%	0.0	98.56%	***LOC102181009***	585	100%	0.0	98.33%
	Human	*TAS2R10*	707	99%	0.0	78.05%	*TAS2R10*	367	100%	3e-124	64.21%
	Dog	*TAS2R10*	830	100%	0.0	80.44%	*TAS2R10*	440	100%	7e-153	73.24%
	Mice	*tas2r5*	367	99%	3e-98	70.15%	-	-	-	-	-

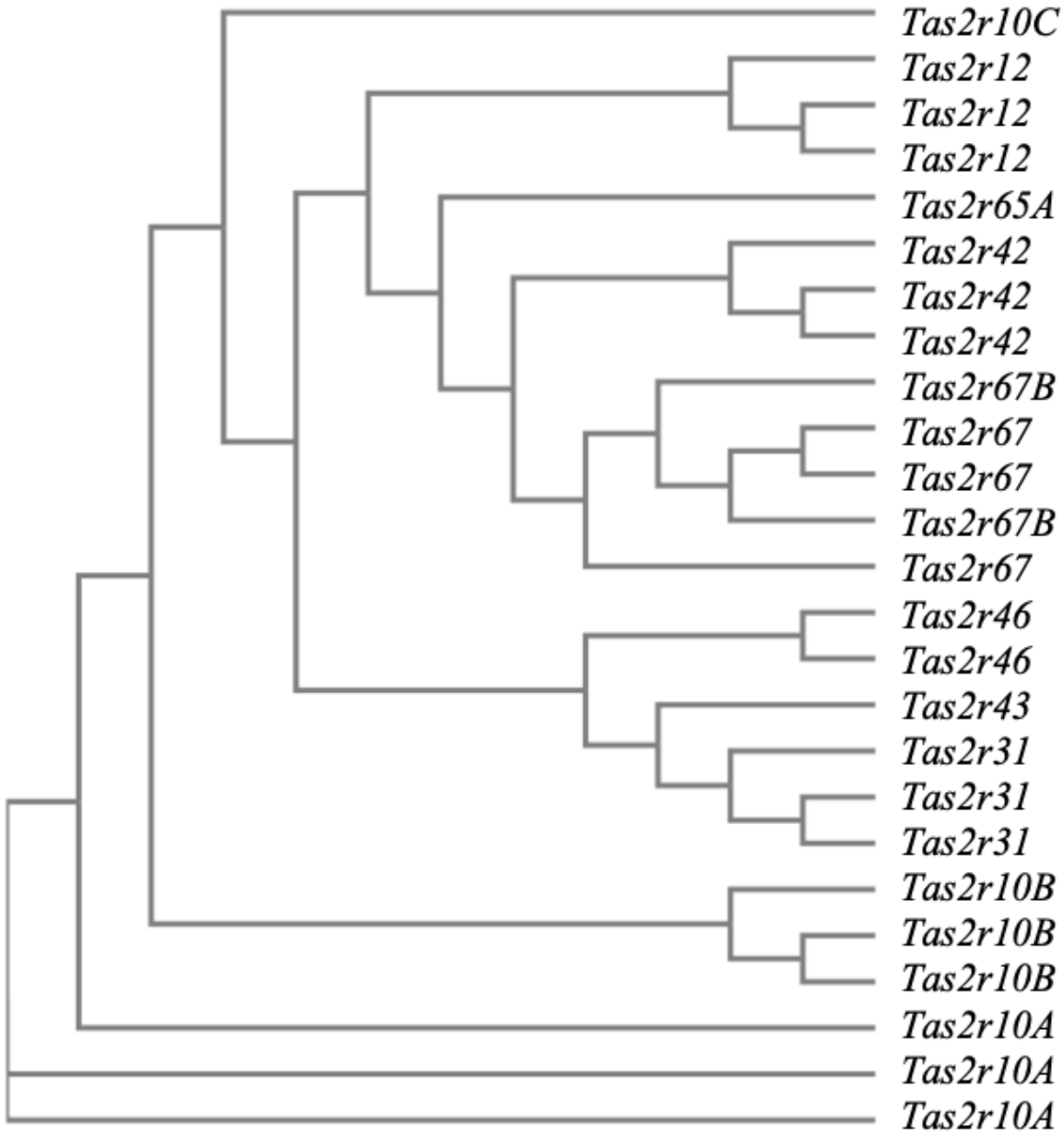


Figure 3.1. Phylogenetic tree of non-annotated Tas2r genes in Cluster 2 for sheep, cattle, and goats.

Table 3.7. Nucleotide sequence percent similarity comparison of non-annotated genes in cattle, sheep and goat. For sizing matrix was divided in half.

Tas2r10C	Cattle10C	100.00	78.39	78.71	79.25	79.22	79.33	79.44	50.73	50.62	50.84	51.38	52.78	53.49
	Cattle10B	78.39	100.00	94.95	95.38	87.56	88.11	87.56	52.86	52.07	52.52	51.14	55.33	55.59
Tas2r10B	Sheep5110	78.71	94.95	100.00	99.35	87.44	88.11	87.56	53.30	52.52	52.97	50.78	55.33	55.81
	Goat0468	79.25	95.38	99.35	100.00	87.67	88.33	87.78	53.19	52.41	52.86	50.42	55.33	55.81
Tas2r10	Cattle10	79.22	87.56	87.44	87.67	100.00	95.33	95.67	53.07	52.84	52.84	49.88	53.97	54.69
	Sheep2269	79.33	88.11	88.11	88.33	95.33	100.00	98.56	53.53	53.30	53.30	50.85	54.42	54.92
	Goat1009	79.44	87.56	87.56	87.78	95.67	98.56	100.00	53.88	53.65	53.65	50.61	54.76	55.37
Tas2r12	Cattle12	50.73	52.86	53.30	53.19	53.07	53.53	53.88	100.00	94.52	94.41	53.87	51.08	52.04
	Sheep4857	50.62	52.07	52.52	52.41	52.84	53.30	53.65	94.52	100.00	99.23	52.40	50.74	51.92
	Goat0198	50.84	52.52	52.97	52.86	52.84	53.30	53.65	94.41	99.23	100.00	52.40	51.19	52.15
Tas2r65A	Cattle65A	51.38	51.14	50.78	50.42	49.88	50.85	50.61	53.87	52.40	52.40	100.00	48.56	49.46
Tas2r46	Goat9923	52.78	55.33	55.33	55.33	53.97	54.42	54.76	51.08	50.74	51.19	48.56	100.00	94.75
	Cattle46	53.49	55.59	55.81	55.81	54.69	54.92	55.37	52.04	51.92	52.15	49.46	94.75	100.00
Tas2r31B	Goat9944	100.00	88.24	87.38	86.92	56.02	55.21	55.21	59.87	58.19	59.50	58.98	58.98	
Tas2r31	Cattle2597	88.24	100.00	96.28	96.30	53.82	53.27	53.49	58.00	56.61	57.27	57.66	56.72	
	Sheep1003	87.38	96.28	100.00	98.03	53.28	52.73	52.95	57.93	56.75	57.30	57.93	56.86	
	Goat9653	86.92	96.30	98.03	100.00	54.15	53.60	53.82	58.11	56.72	57.05	57.78	56.72	
Tas2r42	Cattle0395	56.02	53.82	53.28	54.15	100.00	97.31	96.99	65.15	65.91	65.37	59.08	64.72	
	Sheep42	55.21	53.27	52.73	53.60	97.31	100.00	99.25	64.82	65.26	65.04	64.57	64.39	
	Goat9081	55.21	53.49	52.95	53.82	96.99	99.25	100.00	64.93	65.37	65.15	64.69	64.50	
Tas2r67B	Sheep0742	59.87	58.00	57.93	58.11	65.15	64.82	64.93	100.00	93.59	94.98	95.52	94.66	
Tas2r67	Cattle42	58.19	56.61	56.75	56.72	65.91	65.26	65.37	93.59	100.00	95.95	95.07	95.63	
	Sheep0486	59.50	57.27	57.30	57.05	65.37	65.04	65.15	94.98	95.95	100.00	97.94	98.30	
Tas2r67B	Goat9365	58.98	57.66	57.93	57.78	59.08	64.57	64.69	95.52	95.07	97.94	100.00	98.51	
Tas2r67	Goat9076	58.98	56.72	56.86	56.72	64.72	64.39	64.50	94.66	95.63	98.30	98.51	100.00	

Table 3.8. Proposed annotations of Tas2r genes on Cluster 1 and 2 (**"*"**) for sheep, cattle and goat. Heading abbreviations indicate chromosome location "Ch", number of base pairs the gene contains "BP", number of amino acids the gene contains "AA", and the direction in which the gene is coded "CD".

Species	Gene	Nucleotide	Ch	BP	AA	IL	CD	Proposed
Sheep	TAS2R42		3	930	209	Y	F	42*
	LOC101120486	*(T2R42)*	3	939	312	Y	F	67*
	LOC101120742	*(T2R42 like)*	3	936	311	Y	F	67B*
	LOC101121003	*(31 like)*	3	914	304	Y	F	31*
	LOC101114857	*(T2R7)*	3	912	303	Y	F	12*
	LOC101115110	*(T2R10)*	3	930	309	Y	F	10B*
	LOC101122269	*(T2R10 like)*	3	900	299	Y	F	10*
	9		3	1164	327	Y	F	9
	8		3	929	309	Y	F	8
	7		3	939	312	Y	F	7
Goat	LOC102185981	*(T2R41 like)*	4					41*
	LOC102169081	*(T2R42 like)*	5	931	306	Y	F	42*
	LOC102179076	*(T2R42)*	5	939	312	Y	F	67*
	LOC102169365	*(T2R42 like)*	5	1039	290	Y	F	67B*
	LOC102179923	*(T2R31 like)*	5	915	305	Y	F	46*
	LOC102169653	*(T2R31 like)*	5	918	305	Y	F	31*
	LOC102169944	*(T2R31)*	5	763	254	Y	F	43*
	LOC102180198	*(T2R7)*	5	912	303	Y	F	12*
	LOC102180468	*(T2R10)*	5	930	309	Y	F	10B*
	LOC102181009	*(T2R10 like)*	5	900	299	Y	F	10*
	9		5	935	311	Y	F	9
	8		5	995	331	Y	F	8
	7		5	939	312	Y	F	7
Cattle	LOC100140395	*(T2R42)*	5	3159	309	Y	F	42*
	42		5	932	312	Y	F	67*
	46		5	912	305	Y	F	46
	LOC782957	*(T2R31)*	5	918	305	Y	F	31*
	65A		5	888	295	Y	F	65A
	12		5	930	309	Y	F	12
	10b		5	933	310	Y	F	10B
	10		5	900	299	Y	F	10
	10c		5	930	309	Y	F	10C
	9		5	1272	363	N	F	9
	8		5	930	309	Y	F	8
	7		5	939	312	Y	F	7

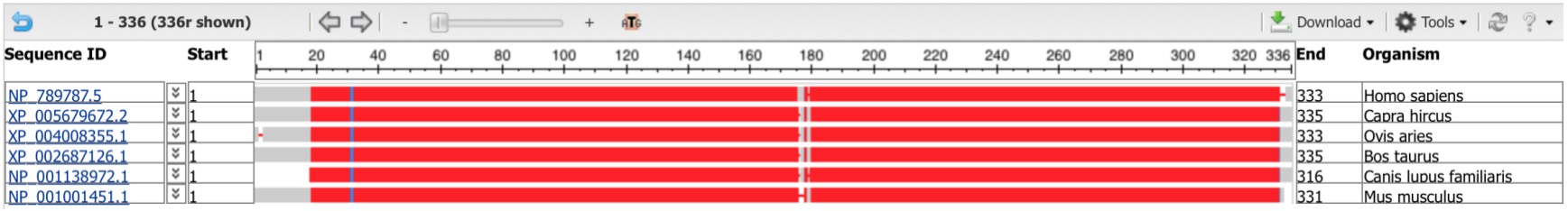


Figure 3.2. Protein alignment results for *TAS2R38* with the "Conservation" option selected. Red depicts highly conserved regions, blue depicts less-conserved regions, and gray depicts regions that not all species express.

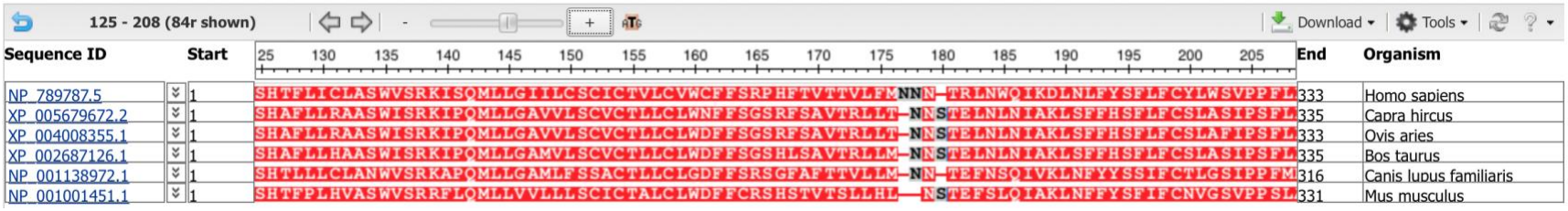


Figure 3.3. Protein alignment results for *TAS2R38* depicting the insertions and deletions found in position 177-180 (dependent upon species).

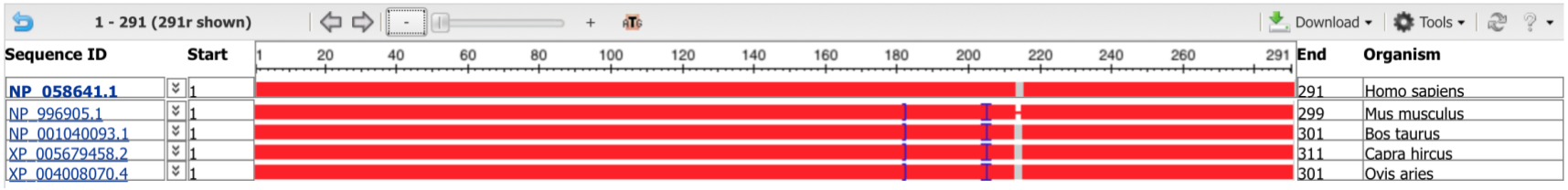


Figure 3.4. Protein alignment for *TAS2R16*. Red designates highly conserved regions, blue "I's" indicate an insertion, and gray indicates regions that not all species express.

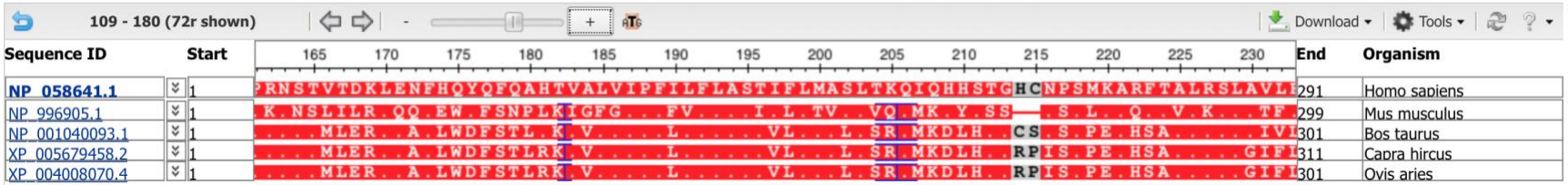


Figure 3.5. Insertions and differences within protein alignment for *TAS2R16* with human set as the master sequence.

Chapter 4: Implications

The research outlined in this thesis stemmed from a very simple question, “can we use sheep to suppress the growth of sagebrush and extend the time period when sagebrush-steppe rangelands have the optimal carrying capacity?” Although a simple question, the answer was far from simple. Sheep are known to browse on sagebrush, but consumption is variable among individual sheep (10 – 40% of diet composition; Snowder et al., 2001). In an effort to better understand factors that drive dietary preference variability in sheep, we investigated PTC avoidance in rams (n = 30; Chapter 2). Results from this trial suggested that sheep exhibit a similar relationship to that observed in humans; whereas PTC concentration increased, acceptance decreased. Because bitterness is often associated with toxins, avoidance of bitter-tasting substances is likely a mechanism in which to avoid ingestion of toxins (Garcia and Hankins, 1975). However, while it has been established that bitter-taste perceptions are variable in humans, it is unknown if bitterness intensity is perceived the same or different among individuals in other species. Furthermore, similar to human haplotypes, we observed variation among individual rams in PTC concentrations at which avoidance occurred and rams tended to form PTC consumption groups, similar to groupings identified in humans (Chapter 2). Taken together, this data suggests that sheep were able to detect bitter-taste and that individual variation in bitterness avoidance may play a role in dietary preferences and intake.

The variation in bitterness intake may translate to foraging preferences while grazing, where sheep with greater tolerance for bitter-taste may consume plants with greater concentrations of bitter tasting compounds, such as the monoterpenoids found in sagebrush. Similarly, humans that have been categorized as non-tasters consume more anti-oxidant rich vegetables with bitter-tasting attributes than tasters (Garcia-Bailo et al., 2009). In humans, variation in bitterness avoidance is known to be driven largely by genetics. In European populations, variations within type two taste receptors (Tas2r) account for 85% of the variability in PTC avoidance (Kim et al., 2003).

Sheep are known to have Tas2r genes, but due to limited research within the sheep genome, several of these genes are non-annotated. Understanding the role that Tas2r genes have on human preferences, traits, and diseases has become of significant importance and likely may be just as important in grazing animals. The completed annotation of the sheep, cattle, and goat Tas2r repertoires described in Chapter 3 will allow for extensive research into the functionality Tas2r genes that may influence diet selection. Identification of Tas2r genes and variation within a species may be used to predict dietary preferences of individual animals. Moreover, it may allow for selection of animals based on their genotype for grazing strategies, which could allow for improvement of rangelands. Similar genetic structures of Tas2r genes among grazing animals suggests that there will be similarity in associated phenotypic traits. Therefore, a discovery relative to Tas2r in sheep would likely also be observed in goat and possibly cattle.

Ongoing research, which will complement the research outlined in this thesis, includes determining whether variations in Tas2r genes in sheep exist, and if so, whether those genetic variations translate into PTC avoidance. To quantify variants of Tas2r genes in sheep, blood was collected, and DNA was isolated from each of the rams used in the PTC trial described in Chapter 2. Primers were developed using NCBI's Primer Blast for each of the 20 known Tas2r genes in sheep and DNA was amplified using polymerase chain reaction (PCR). Amplicons were built and the size of the amplicon was checked using gel electrophoresis. The gel electrophoresis consistently yielded interesting results (Figure 4.1). The length of the some Tas2r genes were different among individuals which suggests that there were likely insertions and/or deletions, but nonetheless, the gels indicated that there was genetic Tas2r genes variants among rams. In humans, PTC avoidance has been linked to the *TAS2R38* gene (Kim et al., 2003). Furthermore, other traits, such as dietary preferences, obesity, and other diseases, have been associated with variations in Tas2r genes in humans (Keller and Tepper, 2004; Wooding et al., 2004). Similarly, it is likely that genetic variations within Tas2r

genes among individuals could translate to differences in bitterness avoidance and/or differences in dietary selection in grazing animals, including sheep.

Another ongoing study, which will also complement the research described in this thesis, was aimed at determining the differences in sagebrush intake in rams using near infrared spectroscopy (NIRS). Using methods outlined by Walker et al. (1996), a NIRS curve for sagebrush was calibrated at the U.S. Sheep Experiment Station by collecting fecal samples collected from sheep (n = 18) fed increasing amounts of sagebrush mixed into a ration of known forages (e.g. grasses). Next, the same rams that were used in the PTC trial described in Chapter 2 were allowed to graze in a sagebrush-steppe pasture for 14 days in October 2018. Fecal samples were collected from each ram prior to grazing and after grazing. The fecal samples will be analyzed for sagebrush content using NIRS with the calibrated sagebrush curve. Results from this trial will provide information on individual preferences for sagebrush in their diets, which may be associated with PTC avoidance and/or Tas2r genomic variations.

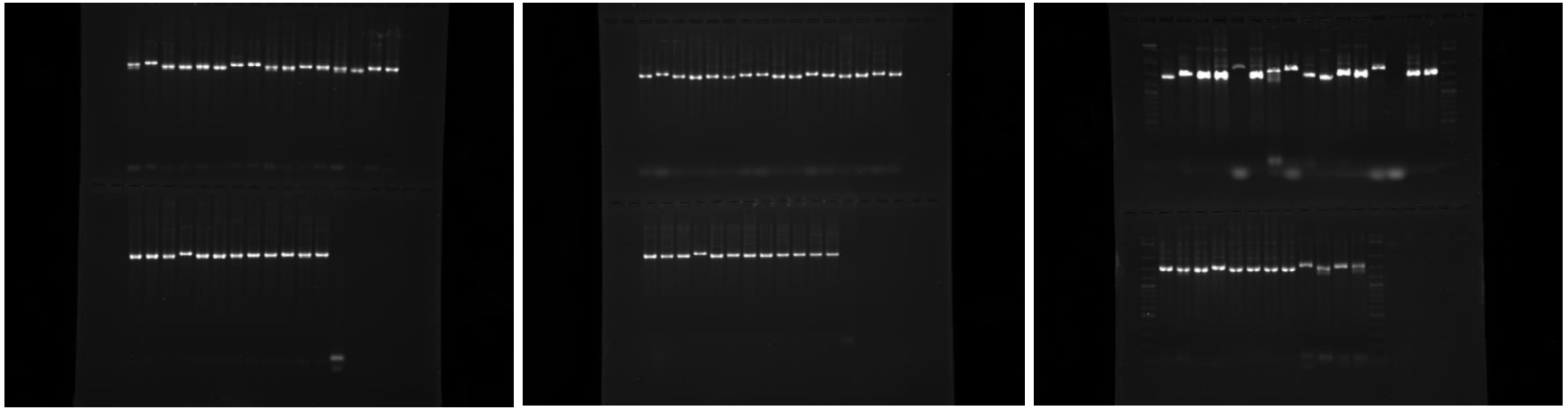
Conducting this series of experiments on the same population of rams has provided a unique opportunity to link genotypic traits (variants in Tas2r genes) with phenotypic traits (avoidance of PTC and willingness to consume sagebrush). It is possible that a similar correlation between *TAS2R38* variants and PTC avoidance observed in humans will also exist in sheep. The *TAS2R38* gene, or other Tas2r genes, may also be associated with willingness to consume sagebrush. Because Tas2r genes detect specific ligands, depending on the ability to bind to the G-protein found within each receptor, it is likely that one or several Tas2r genes play a role in bitter-taste and toxin detection in sheep. Because sagebrush contains bitter-tasting monoterpenoids, determining variants in Tas2r gene may lead to the ability to predict consumption of sagebrush by sheep. Furthermore, consumption of sagebrush is moderately heritable (h_2 = 0.28) in sheep, therefore, selection for individuals that consume sagebrush would likely be passed down to offspring and shift dietary preferences of the

flock over time. It is of interest whether Tas2r genes, PTC avoidance, and individual preference for sagebrush can be linked.

Although the research described in this thesis focuses primarily on sagebrush, the implications could be endless. Sheep have notoriously been utilized for targeted grazing of noxious weeds, shrubs, and other plants. The ability to select individuals that are uniquely suited for targeted grazing would be an important tool for rangeland management. Additionally, selecting for animals that readily consume low-quality forages would allow for increased use of geographic areas that do not support agriculture or habitation by humans. Low-quality forages are found globally, and selection for individuals whose dietary preferences are not to avoid these forages could improve animal production and ecosystem health, especially in third world countries. Moreover, as the human population continues to grow, there is a need to better utilize low-quality forages to produce quality food and fiber on diminishing landscapes.

Finally, utilizing sheep as a grazing tool to *reduce* sagebrush canopy has been suggested to entail long-term and high-intensity grazing applications; however, sheep grazing may be a suitable tool for *suppressing* sagebrush canopy growth and decreasing shrub encroachment on rangelands. It has been well-documented that as sagebrush canopy increases, a reduction in grass and forb production occurs, both of which are key plants for livestock grazing and wildlife habitat (e.g. sage-grouse and deer) (Frischknecht and Baker, 1972).The ability to select for sheep that prefer sagebrush would allow for targeting grazing strategies to maintain optimal sagebrush canopy and extend the time period of greatest carrying capacity within rangelands. Therefore, sheep grazing can be used as an important tool for maintaining healthy sagebrush-steppe rangelands and improving wildlife habitat, which is of great value for livestock producers and land managers in the Intermountain West.

Tables and Figures



TAS2R8 *TAS2R67B* *TAS2R38*

Figure 4.1. Gel electrophoresis results for three type two taste receptors (Tas2r) in sheep (n = 30).

Table 4.1 Primers developed for each gene using NCBI's Primer Blast.

Tas2r gene		Primer	Gene length	Targeted length
16	F	TTTCTGTTGGTGCTGATATTGCAGATGGCTGTGGGCAAAGAG	928	1300
	R	ACTTGCCTGTCGCTCTATCTTCGGAACCTGGTCCCAAACTGG		
3	F	TTTCTGTTGGTGCTGATATTGCCAGCTAACGGTCTGGAGGTC	2841	3110
	R	ACTTGCCTGTCGCTCTATCTTCCAGTAACAGCTTCACCGCCT		
4	F	TTTCTGTTGGTGCTGATATTGCCCCAGGTTCACTTTGGTGGT	1710	1900
	R	ACTTGCCTGTCGCTCTATCTTCCCACAGTCCTGCTGTTCCAA		
5	F	TTTCTGTTGGTGCTGATATTGCAGATTGCAGAAGGGTAAGACCA	2645	3200
	R	ACTTGCCTGTCGCTCTATCTTCTATCTCAAAACAGTCTCCTGACCAC		
38	F	TTTCTGTTGGTGCTGATATTGCGTGGAAGGGCCCATTGATGTA	1002	1382
	R	ACTTGCCTGTCGCTCTATCTTCAGCTTCTGCATCACCCAAGG		
39	F	TTTCTGTTGGTGCTGATATTGCCACACCAGCGCATCCAAAAA	1092	1532
	R	ACTTGCCTGTCGCTCTATCTTCCAGCCCCGGAAATCTTGACT		
40	F	TTTCTGTTGGTGCTGATATTGCTAAACCGGGACTCTTGCCCT	1364	1870
	R	ACTTGCCTGTCGCTCTATCTTCTGACTCTGGGTTAGTGGGGT		
134	F	TTTCTGTTGGTGCTGATATTGCATCCTGGAGGACGGATGGAA	881	1381
	R	ACTTGCCTGTCGCTCTATCTTCTCTGTAAAGGCGGTGTGGAC		
60	F	TTTCTGTTGGTGCTGATATTGCAATTCATGGACAGGCAGCGA	954	1400
	R	ACTTGCCTGTCGCTCTATCTTCTCTTTGGCCACATCAGGTCC		
41	F	TTTCTGTTGGTGCTGATATTGCGAGCTCAGTCACAGACACCC	936	1300

	R	ACTTGCCTGTCGCTCTATCTTCTCCCAAAGGAGAAAGCCCAC		
42	F	TTTCTGTTGGTGCTGATATTGCTGCCGATGATGAATGCACAC	930	1519
	R	ACTTGCCTGTCGCTCTATCTTCGCCTCTTCTCCCAAATACGAGT		
67	F	TTTCTGTTGGTGCTGATATTGCAGTGGGCACATTCACTGCTT	939	1422
	R	ACTTGCCTGTCGCTCTATCTTCTGATGCCAGTGATGCTTGCT		
67B	F	TTTCTGTTGGTGCTGATATTGCTGCCAGCACCAATGATGAGT	936	1536
	R	ACTTGCCTGTCGCTCTATCTTCGGGCATGTCCAAATGATCGTG		
31	F	TTTCTGTTGGTGCTGATATTGCTCCATCCCATAGTAGGGCAC	914	1280
	R	ACTTGCCTGTCGCTCTATCTTCAGACACTTTTTGTTATTAGCTCAGG		
12	F	TTTCTGTTGGTGCTGATATTGCAGCAGTGGCGACACATACAT	912	1412
	R	ACTTGCCTGTCGCTCTATCTTCTGAGAGGTCATCATCACTTCAGG		
10B	F	TTTCTGTTGGTGCTGATATTGCAGGCATTCAGTCTGGGTGTG	930	1370
	R	ACTTGCCTGTCGCTCTATCTTCGGGAGAAACCACTGGCAAGA		
10	F	TTTCTGTTGGTGCTGATATTGCTGGAGGCATCTCTGTCAAGC	900	1350
	R	ACTTGCCTGTCGCTCTATCTTCGGGAGAAACCACTCCAAGGG		
9	F	TTTCTGTTGGTGCTGATATTGCTTTGAAGTCCCTGGCCAACA	1164	1464
	R	ACTTGCCTGTCGCTCTATCTTCTGGTGTGAAGTGTGAACGTGA		
8	F	TTTCTGTTGGTGCTGATATTGCGAGCTTGGAACTTTCGGAGGA	929	1400
	R	ACTTGCCTGTCGCTCTATCTTCGTGCACTTTAGTAGGGGCCA		
7	F	TTTCTGTTGGTGCTGATATTGCGGGACCGACAACTGCATTAC	939	1439
	R	ACTTGCCTGTCGCTCTATCTTCTCCTCTGGCAGTTACTGTTAAGAT		

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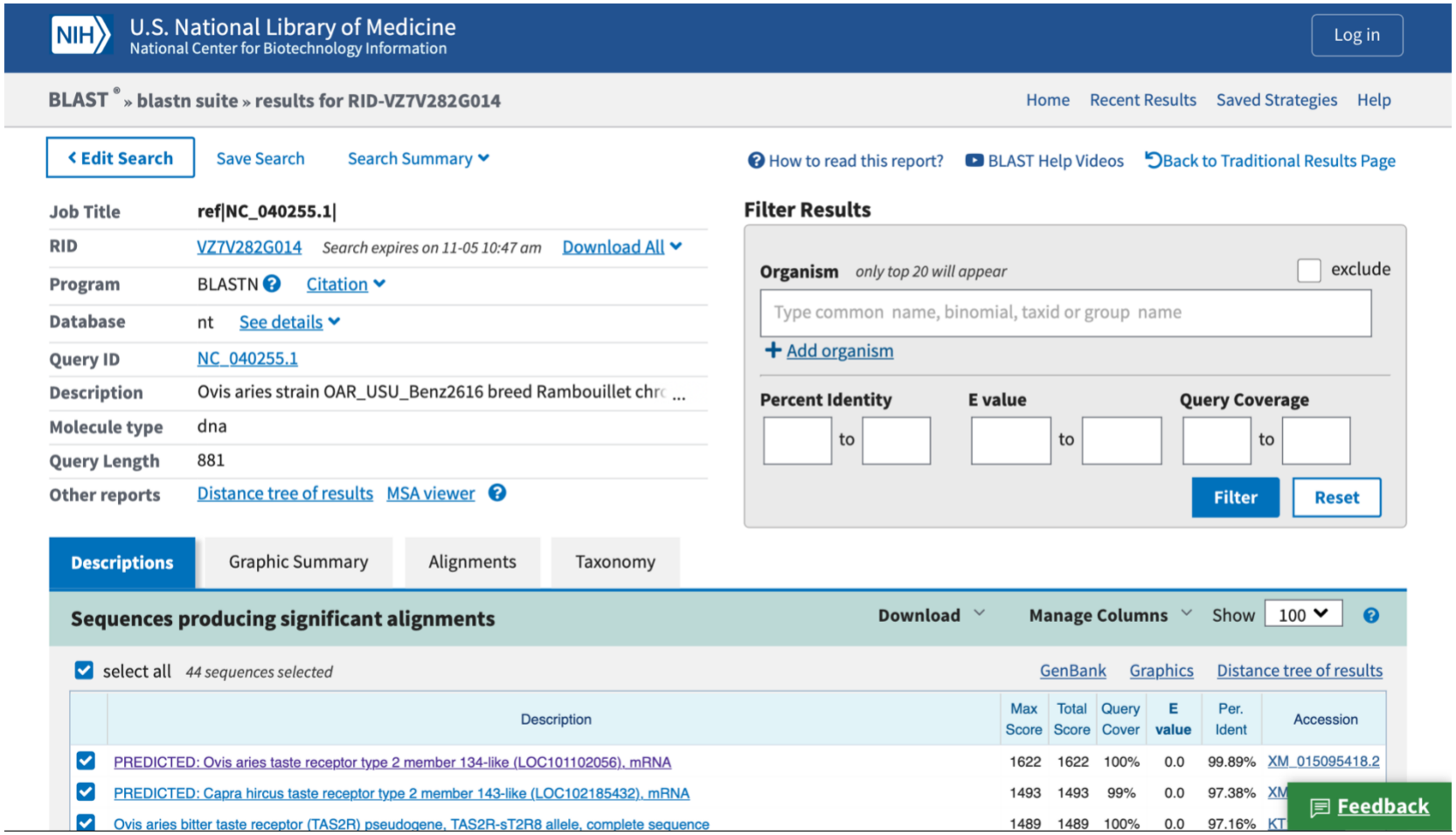
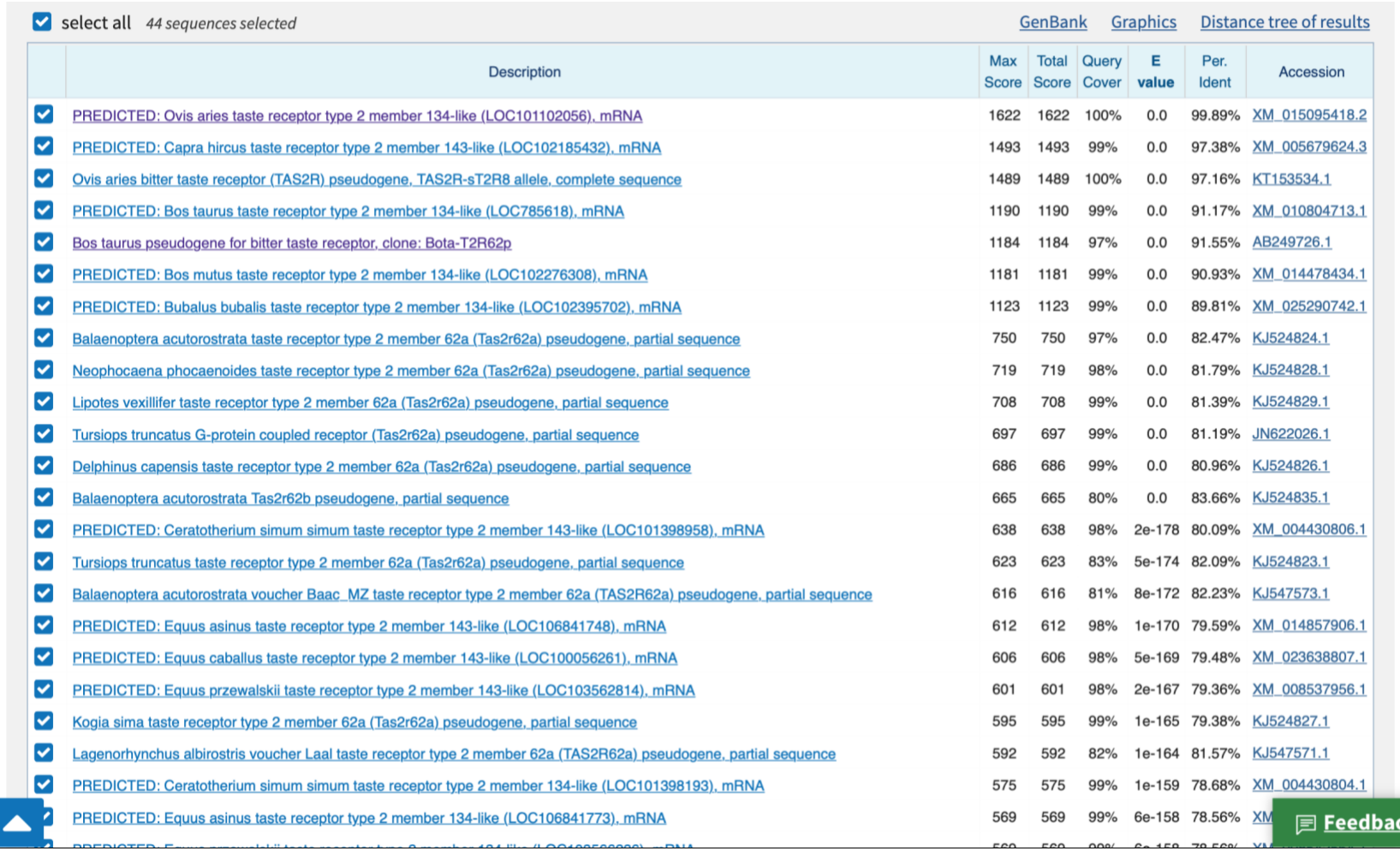
Apendix A



Appendices A.1. Screenshot of nucleotide blast results of sheep LOC101102056.

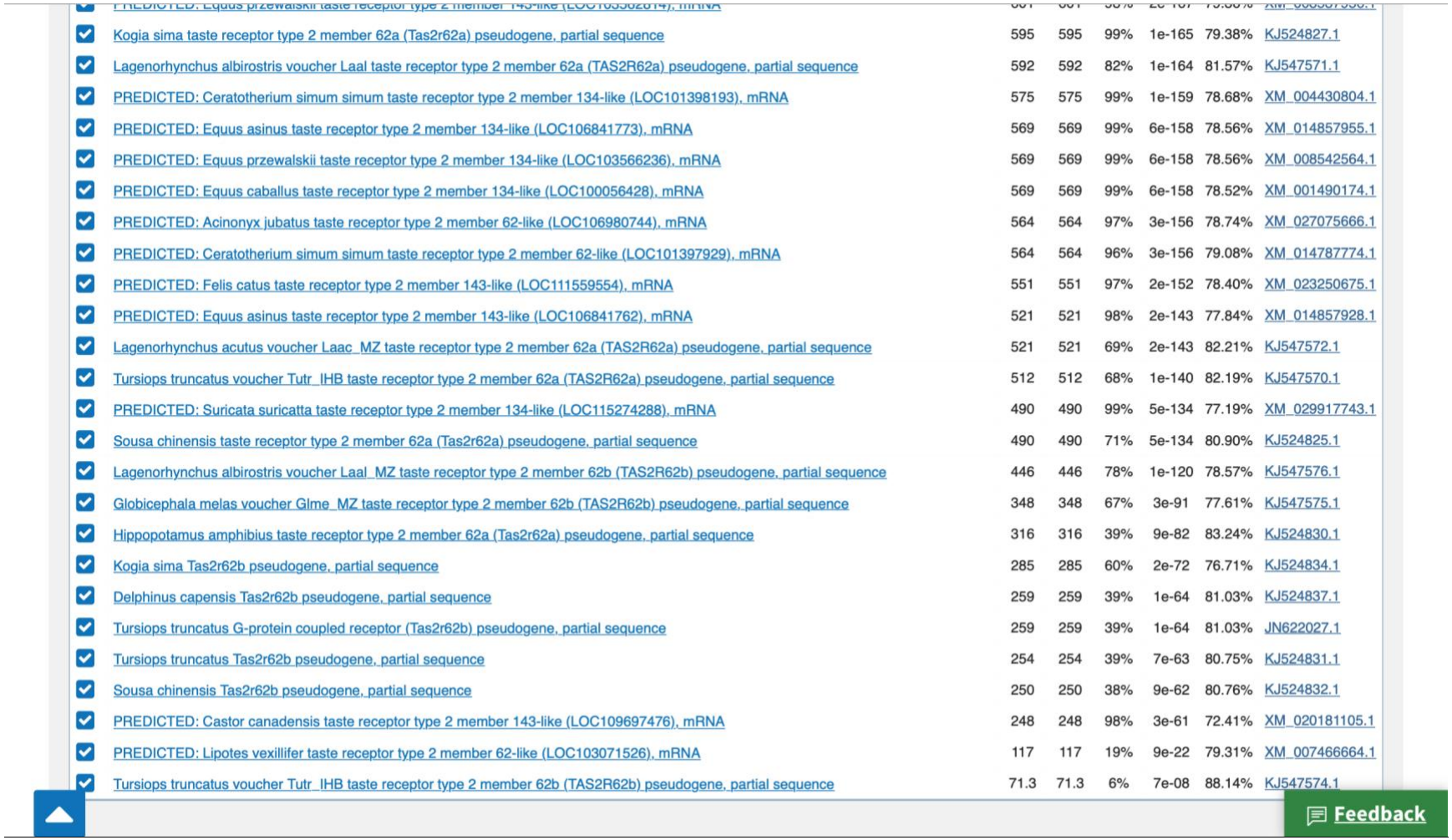


select all 44 sequences selected GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
PREDICTED: Ovis aries taste receptor type 2 member 134-like (LOC101102056), mRNA	1622	1622	100%	0.0	99.89%	XM_015095418.2
PREDICTED: Capra hircus taste receptor type 2 member 143-like (LOC102185432), mRNA	1493	1493	99%	0.0	97.38%	XM_005679624.3
Ovis aries bitter taste receptor (TAS2R) pseudogene, TAS2R-sT2R8 allele, complete sequence	1489	1489	100%	0.0	97.16%	KT153534.1
PREDICTED: Bos taurus taste receptor type 2 member 134-like (LOC785618), mRNA	1190	1190	99%	0.0	91.17%	XM_010804713.1
Bos taurus pseudogene for bitter taste receptor, clone: Bota-T2R62p	1184	1184	97%	0.0	91.55%	AB249726.1
PREDICTED: Bos mutus taste receptor type 2 member 134-like (LOC102276308), mRNA	1181	1181	99%	0.0	90.93%	XM_014478434.1
PREDICTED: Bubalus bubalis taste receptor type 2 member 134-like (LOC102395702), mRNA	1123	1123	99%	0.0	89.81%	XM_025290742.1
Balaenoptera acutorostrata taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	750	750	97%	0.0	82.47%	KJ524824.1
Neophocaena phocaenoides taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	719	719	98%	0.0	81.79%	KJ524828.1
Lipotes vexillifer taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	708	708	99%	0.0	81.39%	KJ524829.1
Tursiops truncatus G-protein coupled receptor (Tas2r62a) pseudogene, partial sequence	697	697	99%	0.0	81.19%	JN622026.1
Delphinus capensis taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	686	686	99%	0.0	80.96%	KJ524826.1
Balaenoptera acutorostrata Tas2r62b pseudogene, partial sequence	665	665	80%	0.0	83.66%	KJ524835.1
PREDICTED: Ceratotherium simum simum taste receptor type 2 member 143-like (LOC101398958), mRNA	638	638	98%	2e-178	80.09%	XM_004430806.1
Tursiops truncatus taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	623	623	83%	5e-174	82.09%	KJ524823.1
Balaenoptera acutorostrata voucher Baac_MZ taste receptor type 2 member 62a (TAS2R62a) pseudogene, partial sequence	616	616	81%	8e-172	82.23%	KJ547573.1
PREDICTED: Equus asinus taste receptor type 2 member 143-like (LOC106841748), mRNA	612	612	98%	1e-170	79.59%	XM_014857906.1
PREDICTED: Equus caballus taste receptor type 2 member 143-like (LOC100056261), mRNA	606	606	98%	5e-169	79.48%	XM_023638807.1
PREDICTED: Equus przewalskii taste receptor type 2 member 143-like (LOC103562814), mRNA	601	601	98%	2e-167	79.36%	XM_008537956.1
Kogia sima taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	595	595	99%	1e-165	79.38%	KJ524827.1
Lagenorhynchus albirostris voucher Laal taste receptor type 2 member 62a (TAS2R62a) pseudogene, partial sequence	592	592	82%	1e-164	81.57%	KJ547571.1
PREDICTED: Ceratotherium simum simum taste receptor type 2 member 134-like (LOC101398193), mRNA	575	575	99%	1e-159	78.68%	XM_004430804.1
PREDICTED: Equus asinus taste receptor type 2 member 134-like (LOC106841773), mRNA	569	569	99%	6e-158	78.56%	XM

Feedback

Appendices A.2. Screenshot of nucleotide blast results of sheep LOC101102056.



PREDICTED: Equus przewalskii taste receptor type 2 member 143-like (LOC103562814), mRNA							
Kogia sima taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	595	595	99%	1e-165	79.38%	KJ524827.1
Lagenorhynchus albirostris voucher LaaI taste receptor type 2 member 62a (TAS2R62a) pseudogene, partial sequence	592	592	82%	1e-164	81.57%	KJ547571.1
PREDICTED: Ceratotherium simum simum taste receptor type 2 member 134-like (LOC101398193), mRNA	575	575	99%	1e-159	78.68%	XM_004430804.1
PREDICTED: Equus asinus taste receptor type 2 member 134-like (LOC106841773), mRNA	569	569	99%	6e-158	78.56%	XM_014857955.1
PREDICTED: Equus przewalskii taste receptor type 2 member 134-like (LOC103566236), mRNA	569	569	99%	6e-158	78.56%	XM_008542564.1
PREDICTED: Equus caballus taste receptor type 2 member 134-like (LOC100056428), mRNA	569	569	99%	6e-158	78.52%	XM_001490174.1
PREDICTED: Acinonyx jubatus taste receptor type 2 member 62-like (LOC106980744), mRNA	564	564	97%	3e-156	78.74%	XM_027075666.1
PREDICTED: Ceratotherium simum simum taste receptor type 2 member 62-like (LOC101397929), mRNA	564	564	96%	3e-156	79.08%	XM_014787774.1
PREDICTED: Felis catus taste receptor type 2 member 143-like (LOC111559554), mRNA	551	551	97%	2e-152	78.40%	XM_023250675.1
PREDICTED: Equus asinus taste receptor type 2 member 143-like (LOC106841762), mRNA	521	521	98%	2e-143	77.84%	XM_014857928.1
Lagenorhynchus acutus voucher LaaC_MZ taste receptor type 2 member 62a (TAS2R62a) pseudogene, partial sequence	521	521	69%	2e-143	82.21%	KJ547572.1
Tursiops truncatus voucher TutrIHB taste receptor type 2 member 62a (TAS2R62a) pseudogene, partial sequence	512	512	68%	1e-140	82.19%	KJ547570.1
PREDICTED: Suricata suricatta taste receptor type 2 member 134-like (LOC115274288), mRNA	490	490	99%	5e-134	77.19%	XM_029917743.1
Sousa chinensis taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	490	490	71%	5e-134	80.90%	KJ524825.1
Lagenorhynchus albirostris voucher LaaI_MZ taste receptor type 2 member 62b (TAS2R62b) pseudogene, partial sequence	446	446	78%	1e-120	78.57%	KJ547576.1
Globicephala melas voucher GlmeMZ taste receptor type 2 member 62b (TAS2R62b) pseudogene, partial sequence	348	348	67%	3e-91	77.61%	KJ547575.1
Hippopotamus amphibius taste receptor type 2 member 62a (Tas2r62a) pseudogene, partial sequence	316	316	39%	9e-82	83.24%	KJ524830.1
Kogia sima Tas2r62b pseudogene, partial sequence	285	285	60%	2e-72	76.71%	KJ524834.1
Delphinus capensis Tas2r62b pseudogene, partial sequence	259	259	39%	1e-64	81.03%	KJ524837.1
Tursiops truncatus G-protein coupled receptor (Tas2r62b) pseudogene, partial sequence	259	259	39%	1e-64	81.03%	JN622027.1
Tursiops truncatus Tas2r62b pseudogene, partial sequence	254	254	39%	7e-63	80.75%	KJ524831.1
Sousa chinensis Tas2r62b pseudogene, partial sequence	250	250	38%	9e-62	80.76%	KJ524832.1
PREDICTED: Castor canadensis taste receptor type 2 member 143-like (LOC109697476), mRNA	248	248	98%	3e-61	72.41%	XM_020181105.1
PREDICTED: Lipotes vexillifer taste receptor type 2 member 62-like (LOC103071526), mRNA	117	117	19%	9e-22	79.31%	XM_007466664.1
Tursiops truncatus voucher TutrIHB taste receptor type 2 member 62b (TAS2R62b) pseudogene, partial sequence	71.3	71.3	6%	7e-08	88.14%	KJ547574.1

Appendices A.3. Screenshot of nucleotide blast results of sheep LOC101102056.